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Time-Course of Nigrostriatal Degeneration in a Progressive MPTP-Lesioned Macaque Model of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is characterized by a progressive loss of substantia nigra pars compacta (SNc) neurons. The onset of clinical symptoms only occurs after the degeneration has exceeded a certain threshold. In most of the current 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate models, nigrostriatal lesions and the onset of PD symptoms are the result of an immediate neuronal degeneration in the SNc caused by acute injection of the toxin. In order to develop a model that more closely mimics the degeneration pattern of human PD, we eventually established a protocol that produces a progressive parkinsonian state by treating monkeys repeatedly with MPTP for 15 ± 2 d. Mean onset of parkinsonian symptoms occurred after 13.2 d of treatment. At this time, 56.8 ± 6.3% of tyrosine hydroxylase immunoreactive neurons and 75.2 ± 6.2% of Nissl-stained cells remained in the SNc. Striatal dopamine transporter (DAT) binding and dopamine (DA) content decreased to 19.7 ± 4.9% and 18.2 ± 5.6% of untreated monkeys. Parallel ^{123}I-PEI single-photon emission computed tomography (SPECT) imaging in living animals showed a similar decrease in striatal DAT binding. In this article, we examine how this and other chronic MPTP models fit with human pathology.

Index Entries: Threshold for symptom appearance; kinetics of nigral degeneration; chronic MPTP model; substantia nigra; striatum.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that afflicts 1% of the population over the age of 55 (Hoehn and Yahr, 1967). First described by James Parkinson

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(1817), PD is a syndrome that is characterized by tremor, rigidity, postural abnormalities and bradykinesia. The main pathological finding of PD is the loss of pigmented dopamine (DA) neurons in the substantia nigra pars compacta (SNc) (Hassler, 1938; Ehringer and Hornykiewicz, 1960).

The cause of the ongoing nigral neuronal cell death in PD and the subsequent clinical deterioration remains a mystery. It has been hypothesized that progression in PD is the result of linear age-related cell attrition superimposed upon a SNc that is already damaged by transient exposure to a previous insult (Koller et al., 1991). Alternatively, the onset and progression of idiopathic PD may represent a novel, ongoing degenerative process (McGeer et al., 1988) with an exponential decay (Fearnley and Lees, 1991). Until recently, the presymptomatic phase was believed to last at least 20 yr (Hoehn and Yahr, 1967; Vingerhoets et al., 1994). However, Fearnley and Lees proposed 4.7 yr (1991), and Morrish et al., suggested 3.1 yr (1996). Although the length of the period preceding the appearance of clinical signs remains a matter of debate, it is generally accepted that the onset of parkinsonian symptoms occurs when dopaminergic neuronal death exceeds 70–80% of striatal nerve terminals and 50–60% of SNc pericarya (Bernheimer et al., 1973; Riederer and Wuketich, 1976). The concept of a threshold for the onset of symptoms is widely accepted, and is based on extrapolating measurements of decreased striatal DA content in human postmortem tissue (Hornykiewicz and Kish, 1987) or backward calculation of progression seen in human in vivo imaging studies (Morrish et al., 1996). However, it has never been determined experimentally in nonhuman primates. In most of the present 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-nonhuman primate models, nigrostriatal lesion is achieved by a single bolusinjection of the toxin, either in the carotid artery (Bankiewicz et al., 1986) or intravenously (Chiueh et al., 1985). Either procedure leads to an immediate degeneration of nigral DA neurons and the onset of PD symptoms. In humans, PD progresses over a period of years, and the onset of symptoms occurs only after the nigrostriatal degeneration has exceeded a critical threshold. Thus, our recent research focused on the development of a chronic model of experimental parkinsonism that more closely mimics the progressive degeneration of human PD. Finally, we established a protocol that produces a progressive parkinsonian state by treating monkeys repeatedly with MPTP for a period of 15 ± 2 d (Bezard et al., 1997; Bezard et al., 2000; Bezard et al., 2001c; Bezard et al., 2001a).

Time-Course of Nigrostriatal Degeneration After Chronic MPTP Treatment

The kinetics of nigrostriatal degeneration in this model and the critical thresholds associated with the symptom appearance were further validated by evaluating striatal DA content and metabolism, DA transporter (DAT) binding in striatal sections, striatal DA receptor (DAR; D_1-like and D_2-like subtypes) binding, and the number of both tyrosine hydroxylase immunoreactive (TH-IR) and Niss1-stained neurons in the SNc. Low doses of MPTP (0.2 mg/kg) were administered daily until the appearance of PD symptoms, which occurred on average at d 13.2 with a slowing of general activity and mild flexed posture. Thereafter, motor symptoms progressed until d 25, and monkeys became increasingly bradykinetic, with a severe flexed posture, increasing limb rigidity and decreasing vocalization. Their movements were less accurate, as when reaching for fruits, and there were occasional episodes of freezing. Both, postural and some resting tremors were observed. At d 13.2, 56.8 ± 6.3% of TH-IR and 75.2 ± 6.2% of Nissl-stained neurons remained in the SNc (Fig. 1A). In parallel, DAT binding in striatal sections (Fig. 1B) and DA content decreased to 19.7 ± 4.9% and 18.2 ± 5.6% of D0 values, and striatal D1- and D2-binding were similar to D0 values (Bezard et al., 2001b). In addition to the DAT binding

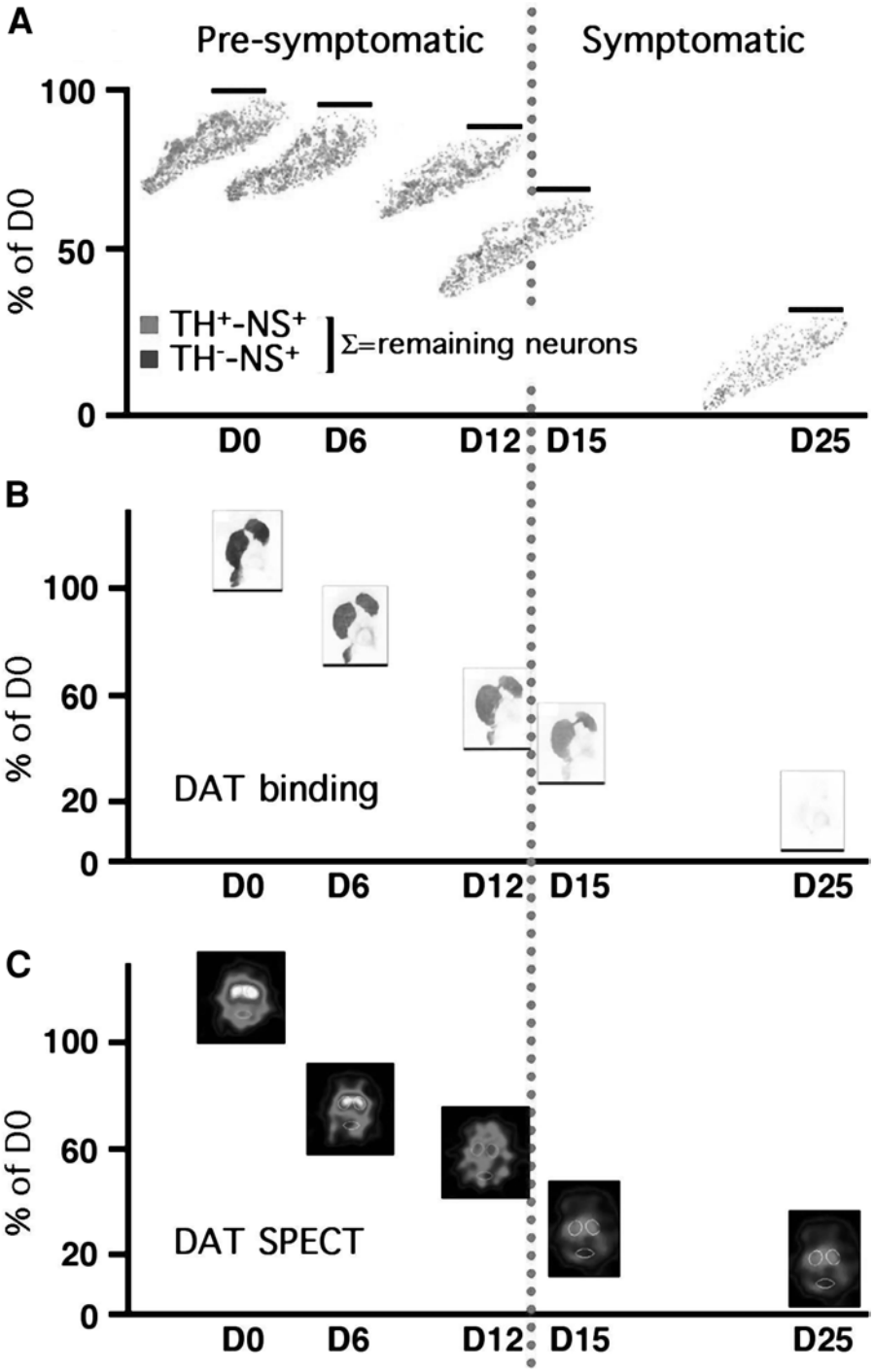

Fig. 1. Time-course of nigrostriatal degeneration. **(A)** Examples of cell counting maps showing the typical patterns of degeneration in the SNc (n = 5 at d 0, d 6, d 12, d 15, and d 25). The number of TH-IR and Nissl-stained neurons in the SNc were counted in one representative section that corresponded to a median plane of the SNc by one examiner who was blinded to the experimental condition. TH-IR neurons are shown in red, and the blue symbols represent the Nissl-stained cells that were not TH-positive. The horizontal line above each map indicates the mean percentage of surviving cells (e.g., Nissl-stained). Note the selective disappearance of the dorsal tier of the SNc with time. **(B)** Representative examples of DAT-binding autoradiographs showing the progression of striatal denervation at the caudal level of the same animals. Note the homogenous degeneration and the severe lesion in the d 25 group. The horizontal line under each example indicates the mean percentage of DAT binding in striatal sections. Nonspecific binding is shown on the bottom left corner of the figure. **(C)** Representative examples of ^{123}I-PE2I SPECT as a marker of DAT binding in living animals during disease progression between d 0 and d 25 (n = 2). In agreement with the DAT binding in striatal sections, there is a homogenous degeneration and severe lesion in the d 25 group. The inferior border of each image corresponds to the mean percentage of striatal ^{123}I-PE2I binding potential, as determined by Logan's graphical method.

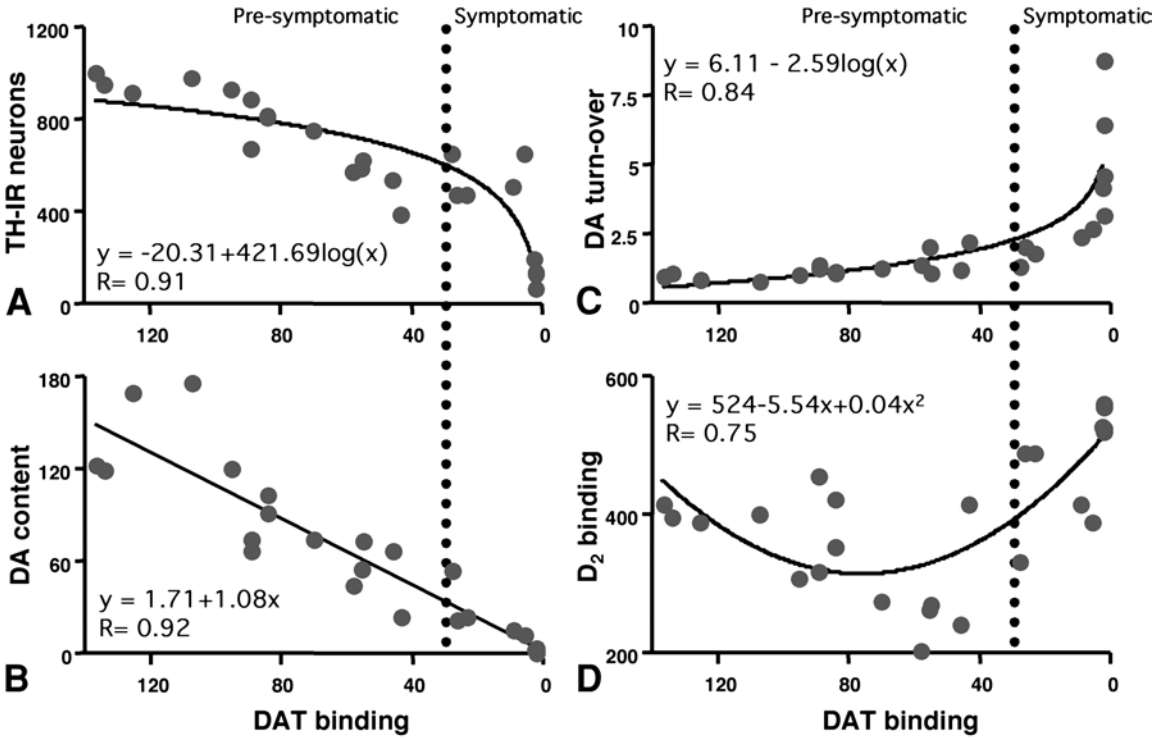

Fig. 2. Relationship between various parameters of nigrostriatal degeneration (n = 5 at d 0, d 6, d 12, d 15, and d 25). Each dot corresponds to one animal. **(A)** Correlation between TH-IR and DAT binding in striatal sections. Initially, MPTP treatment induces a pronounced decrease of DAT binding in striatal sections, whereas the decline in nigral TH-IR neurons is less dramatic. Note the accelerated decrease of TH-IR neurons after the onset of PD symptoms. **(B)** Linear relationship between striatal DA content and DAT binding in striatal sections indicating progressive degeneration of striatal DA terminals. **(C)** DA turnover and DAT binding in striatal sections are logarithmically related. A dramatic increase of DA turnover only occurs after onset of PD symptoms. **(D)** Note the quadratic relationship between striatal D_2-binding and DAT binding in striatal sections. The initial decrease in D_2 DAR binding mainly reflects the disappearance of striatal DA terminals as indicated by decreasing DAT binding in striatal sections, whereas the subsequent increase represents an upregulation of postsynaptic D_2 DAR.

in striatal sections, sequential ^{123}I-PE2I single-photon emission computed tomography (SPECT) acquisitions was performed allowing monitoring of disease progression within the same animal by evaluating striatal DAT binding in living animals (Fig. 1C). In agreement with the DAT binding in striatal sections, the mean distribution volume calculated according to Logan's graphical method was significantly decreased from d 6 onward, as when animals presented as clinically normal (Prunier et al., 2003).

The relationship between the number of TH-IR neurons and DAT binding in striatal sections is best represented by a logarithmic equation:

$$y = -20.31 + 421.69\log(x),\ r = 0.91,\ p < 0.05 \quad (1)$$

As shown in Fig. 2A, within the presymptomatic period, the decrease of DAT binding in striatal sections was more pronounced than the degeneration of TH-IR neurons, which in turn was dramatically accelerated after the

onset of PD symptoms between d 13.2 and d 25. This dissociation suggests that within the applied experimental protocol, SNc neurons may lose the functional integrity of their terminals in early stages while their cell bodies remain unaffected, as reflected by the dramatic decrease in striatal DAT binding and unaltered TH expression. This has also been described in MPTP intoxicated mice (Gross et al., 2003). A mechanism to explain this dissociation may be the so-called "dying back"—an axon of an unhealthy neuron progressively degenerates over a period of weeks or even months, beginning distally and then spreading toward the cell body (Raff et al., 2002). Raff and colleagues (2002) hypothesized that "dying back" may be caused by an activation of a self-destruct program in the distal parts of an axon in response to an external stressor. Subsequently, the nature, extent, and time-course may determine whether the neuron undergoes apoptotic cell death or activates a caspase-independent, axonal self-destruct program to disconnect the axon from its target cell. However, with the onset of PD symptoms, the degeneration of TH-IR neurons is accelerated. Changes in DA content and DAT binding in striatal sections are linearly correlated and are both markers of ongoing degeneration of striatal DA terminals:

$$y = 1.71 + 1.08x,\ r = 0.92,\ p < 0.05 \text{ (Fig. 2B)} \quad (2)$$

The relationship between striatal DA turnover and DAT binding in striatal sections is characterized by a logarithmic correlation:

$$y = 6.11–2.59\log(x),\ r = 0.84,\ p < 0.05 \text{ (Fig. 2C)} \quad (3)$$

Although there is a dramatic decrease of DAT binding in striatal sections, DA turnover increases significantly only at d 25, in full-stage parkinsonism. This underscores the need for pronounced DA depletion before any increase in DA turnover can be observed (Bernheimer et al., 1973; Elsworth et al., 2000). The time-course of D_2-binding cannot be described by a simple equation. The relationship between D_2- and DAT binding in striatal sections is quadratic:

$$y = 524 – 5.54x + 0.04x^2,\ r = 0.75,\ p < 0.05 \text{ (Fig. 2D)} \quad (4)$$

This implies a synergistic action of two first-order processes. Since D_2 DAR are located pre- and postsynaptically, the initial decrease in D_2 DAR binding mainly reflects the disappearance of striatal DA terminals, as indicated by decreasing DAT binding in striatal sections, whereas the subsequent increase represents an upregulation of postsynaptic D_2 DAR.

How Do These Findings Fit With Human PD?

According to clinical studies in human PD, symptom appearance would require a 70–80% loss of striatal terminals and a 70–90% depletion of striatal DA, as well as a 50–60% loss of nigral DA neurons (Bernheimer et al., 1973; Riederer and Wuketich, 1976). In the present chronic MPTP model, the depletion of striatal markers fits with these predictions, and the nigral threshold is lower than expected (Table 1). However, Fearnley and Lees determined a threshold of 31% DA cell loss in human PD (1991), and German and colleagues reported a decrease of 46% in mildly symptomatic MPTP-treated Macaca fascicularis (German et al., 1988a). In accordance with previous reports (German et al., 1988b), the general gradient loss we observed began in the whole dorsal tier of the SNc, and thereafter spread to its ventral tier, where only few TH-IR neurons remained detectable in the fully parkinsonian state (d 25, Fig. 1A) (German et al., 1996). This suggests that the present dynamic MPTP model would correspond to human PD in terms of similar thresholds for symptom appearance. In accordance with neuropathological findings in human MPTP-induced parkisonism (Langston et al., 1999), but in contrast to patients with idiopathic PD, no Lewy bodies were found in surviving SNc neurons. However, the present model is not intended to determine mechanisms of cellular degeneration of PD, but to

Table 1
Kinetics of Nigrostriatal Degeneration

	D 0	D 6	D 12	D 13.2	D 15	D 25
TH-ir	100.0	82.4	62.5	56.8	52.5	14.2
Nissl staining	100.0	97.9	93.3	75.2	71.6	34.7
DAT binding	100.0	69.8	40.6	19.7	18.3	2.3
DA content	100.0	58.3	41.8	18.2	14.2	2.0
DA metabolites/ DA ratio	100.0	130.4	148.3	188.2	232.5	559.3
D_1-like binding	100.0	103.7	95.5	98.3	101.2	91.4
D_2-like binding	100.0	95.7	68.8	93.3	114.9	140.6

Time-course of nigrostriatal degeneration after chronic MPTP treatment between D0 and D25 in the SNc (TH-IR, Nissl staining) and the putamen (DAT binding in striatal sections, DA content, DA metabolites/DA ratio, D_1-binding and D_2-binding) according to Bezard et al. (2001). Values are displayed as percentage of D0. Mean onset of PD symptoms occurs at d 13.2. The given numbers for d 13.2 were calculated from best-fit regression equations.

correlate biochemical and behavioral parameters with a defined extent of nigrostriatal degeneration. Moreover, all current animal models of PD, including the recently proposed rotenone model (Betarbet et al., 2000; Höglinger et al., 2003), have their limitations in the study of the underlying etiology of PD.

Temporospatial Lesion Progression and Nature of the Initial Pathological Event

Acute administration of high doses of MPTP produces acute uniform striatal dopaminergic denervation both in monkeys (Perez-Otano et al., 1994) and humans (Snow et al., 2000). A single low-dose or chronic low-dose regimen of MPTP intoxication produces a greater depletion of dopaminergic markers in the putamen than in the caudate nucleus (Irwin et al., 1990; Moratalla et al., 1992), which is in agreement with the neuropathological findings in PD (Kish et al., 1988; Brooks et al., 1990). Damier and colleagues suggested that the temporospatial lesion progression accounts for the differences in the pathogenesis of MPTP-induced parkinsonism and PD (Damier et al., 1999b). They identified compartmental subdivisions within the SNc (Damier et al., 1999a), each of them distinctively affected by the progression of the disease (Damier et al., 1999b; Hirsch et al., 1988). Based on evidence that suggests a within-SNc origin of the pathological process (Hirsch, 1999), they hypothesized that different subregions would have different projection zones, leading to a gradient of DA depletion with a higher loss in dorsal and caudal parts of the putamen than in the caudate nucleus. Since the active metabolite of MPTP is taken up by DAT (Gainetdinov et al., 1997), a within-striatum trigger would lead to a more uniform striatal denervation. Thus, uniformity of lesion could reflect the fundamental difference between human disease and its closest animal model—e.g., the nature of a possible initial pathological event to trigger PD.

What Are the Lessons Taught by Chronic MPTP Nonhuman Primate Models?

Other chronic MPTP nonhuman primate models of PD have been proposed. For instance, *papio papio* baboons were chronically

treated with MPTP either at weekly intervals for 20–21 mo or daily for 5 d followed 5–6 mo later by weekly injections for 5–21.5 mo. Both regimens invariably resulted in the appearance of a progressive and irreversible parkinsonian syndrome. In some animals, resting tremor and rigidity were initially restricted to one side, and became bilateral within a few months of treatment. The histopathological evaluation showed uneven striatal DA fiber loss and a neuronal depletion in the SNc similar to that in PD (Varastet et al., 1994; Hantraye et al., 1993). Moreover, there was an intriguing aggregation of alpha-synuclein in degenerating nigral neuronal-cell bodies (Kowall et al., 2000), suggesting that the occurrence of Lewy bodies depends on a slow, degenerative process. However, one major limitation of this model is the lack of warranted reproducibility in the timing of the onset of PD. This may be advantageous in reflecting the heterogeneity in human PD, but makes experimental studies extremely ambitious. Schneider and Kovelowski have introduced another chronic MPTP nonhuman primate model of PD for the purpose of studying cognitive deficits in PD. In this model, macaques received chronic low-dose MPTP injections over a period of months, producing extensive DA depletion and clear deficits in a delayed response and alternation task reminiscent of frontal-lobe dysfunction, but retained the ability to correctly perform visual pattern discrimination and showed little or no discernible motor disturbances (Schneider and Kovelowski, 1990; Schneider, 1990). Furthermore, they demonstrated that changes in striatal neuropeptide expression—but not the extent of DA depletion and the degree of motor impairment—were critically dependent on the period of MPTP administration (Schneider et al., 1999). This further raises the question of whether these acute and chronic models have anything in common with progressive degeneration in human PD. They are undoubtedly the best available, but the nigrostriatal lesion is achieved by injection of a toxin without accounting for the multitude of intermingling pathogenetic processes in humans. This makes the research for new animal models as important and as difficult as it has always been. Before considering new models of PD, one should address the question of whether PD is the result of an event or a process (Calne, 1994; Schulzer et al., 1994). The characteristic of an event is the transient occurrence that kills some neurons while damaging others. This may lead to an increasing clinical deficit over time, because damaged neurons may remain functional to a varying extent and substantial duration before they undergo premature death. In contrast, a process describes a mechanism in which healthy neurons are continuously undergoing active destruction over a prolonged course. However, both mechanisms are not mutually exclusive, and it is common for a process to be initiated by an event, which is a remarkable conclusion in view of the bulk of evidence that human PD can result from an event or a process. Moreover, both mechanisms are mathematically indistinguishable (Calne, 1994; Schulzer et al., 1994; Lee et al., 1994). How could this reconcile with new animal models of PD? Is it possible to establish experimental protocols without a thorough understanding of the underlying mechanisms of progressive neuronal death in human PD? One way out could be the use of a toxin that acts as an initial event and which then triggers mechanisms such as inflammation, known to be relevant in the pathogenesis of PD (Hirsch et al., 1998), to maintain progressive degeneration over a longer period of time. This is exactly one of the hypotheses of MPTP action in humans (Langston et al., 1999). Although approx 300 individuals have been exposed to MPTP, only a few developed parkinsonism (Langston et al., 1983). This suggests that the initial event of MPTP exposure is not unrelentingly followed by mechanisms that maintain progressive degeneration.

Conclusions

The classic experimental approach in which the normal situation is compared to the fully

lesioned condition can be complemented by the use of dynamic models that more closely resemble the evolution of the disease (Bezard and Gross, 1998). However, the obvious limitations of all animal models of PD described make research as important and as difficult as before. However, this and other chronic models have made it possible to define the threshold of nigrostriatal degeneration that is mandatory for symptom appearance, and may therefore provide new insights into the pathophysiology of PD.

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