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ORIGINAL ARTICLE

MPTP Induces Systemic Parkinsonism in Middle-Aged Cynomolgus Monkeys: Clinical Evolution and Outcomes

Feng Yue[1,2] · Sien Zeng[3] · Rongping Tang[4] · Guoxian Tao[4] · Piu Chan[1,2]

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Abstract In this study, we developed a systemic PD model in middle-aged cynomolgus monkeys using individualized low-dose MPTP, to explore effective indicators for the early prediction of clinical outcomes. MPTP was not stopped until the animals showed typical PD motor symptoms on days 10 to 13 after MPTP administration when the Kurlan score reached 10; this abrogated the differences in individual susceptibility to MPTP. The clinical symptoms persisted, peaking on days 3 to 12 after MPTP withdrawal (rapid progress stage), and then the Kurlan score plateaued. A Kurlan score at the end of the rapid progress stage >15 reflected stable or slowly-progressive PD, while a score <15 indicated spontaneous recovery. The entire clinical evolution and outcome of the systemic PD model was characterized in this study, thus providing options for therapeutic and translational research.

Keywords Parkinson's disease · Cynomolgus monkeys · MPTP · Motor behavior · Spontaneous recovery · Clinical evolution

✉ Piu Chan
pbchan@hotmail.com; pbchan90@gmail.com

[1] Department of Neurobiology, Beijing Institute of Geriatrics, Xuanwu Hospital of Capital Medical University, Beijing 100053, China

[2] Key Laboratory on Parkinson's Disease, Beijing 100053, China

[3] Department of Pathology, Guilin Medical College, Guilin 541001, China

[4] Wincon TheraCells Biotechnologies Co., Ltd., Nanning 530003, China

Introduction

Studies of the pathogenesis and treatment of Parkinson's disease (PD) require a clinical or experimental model with typical manifestations of consistent, stable, and chronic disease. The non-human primate model of PD induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is considered to be the most effective tool for such studies [1–6]. After administration of MPTP to specifically damage the nigral dopaminergic neurons, the primate manifests typical behavior together with biochemical and histological changes similar to those of primary PD. The behavioral symptoms, resting and kinetic tremor, bradykinesia, reduced locomotion, rigidity, and postural instability, are similar to the manifestation of PD and can be treated effectively with levodopa [7–14].

However, the MPTP-induced model of PD in non-human primates has the following limitations, which restrict its application [14–24]: (1) the large individual differences in response to a similar dosage of MPTP prevent easy comparison across different studies; (2) animals with mild to moderate injury show spontaneous recovery, which is inconsistent with the progressive features of severe PD; there is no scope for effective prediction of spontaneous recovery in the model; and previous studies focused on simulation of different PD stages, with a lack of comprehensive assessment of the clinical evolution and outcome of the entire process; and (3) the supply of old monkeys is limited, so most models have used younger monkeys although PD mainly occurs in middle-aged individuals. Previous studies have shown that the possibility of spontaneous recovery is minimal after creation of the model in older monkeys compared with in the young [24].

So, in this study, middle-aged cynomolgus monkeys were selected to establish a systemic PD model using

MPTP in order to clearly and accurately enable the diagnosis of individuals in the pre-motor period of PD before the manifestation of typical symptoms and the assessment of outcomes of MPTP with levodopa intervention.

Materials and Methods

Experimental Animals

Five healthy middle-aged cynomolgus monkeys (*Macaca fascicularis*) were used in this study. Four females (CF24, CF04, CF36, and CF16) and one male (CM05) aged 9.1–15.3 years (mean 10.81 ± 2.54) were supplied by Guangxi Grandforest Scientific Primate Company after routine quarantine and physical examination. All animals exhibited intact normal behavior without physical impairment. The experiments were performed in a primate facility (Wincon TheraCells Biotechnologies Co., Ltd.) accredited by the Association for Assessment and Accreditation of Laboratory Animal Care with the feeding room temperature at 22–28 °C and a relative humidity of 30%–75% under an alternating 12-h light/dark cycle. Water was supplied ad libitum. Animals were fed twice daily and supplemented with fresh fruit and vegetables once daily at noon. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

MPTP Administration

Atropine (0.04 mg/kg, intramuscular) was injected 30 min before anesthesia with ketamine (4–5 mg/kg, intramuscular). Fifteen minutes later, 0.2 mg/kg MPTP-HCl (Sigma, St. Louis, MO) was slowly injected via a lower extremity vein at 1 mL/min once per day until the apperance of typical PD manifestation [14, 27, 28]. The animals failed to eat on their own on the second day after MPTP injection, and therefore were fed with liquid food via gastric tube twice a day until the end of the experiment.

Levodopa Therapy

The animals were orally treated with levodopa at 5 months post MPTP injection. Madopar (levodopa:benserazide, 4:1; 250 mg/tablet, Roche, Vaud, Switzerland) was initially given at 62.5 mg/kg per day. A week later, the dose was increased to 125 mg/kg per day (1/2 tablet, fed via gastric tube twice a day) for three weeks.

Behavioral Assessment

Video Recording and Clinical Rating

Animals were transferred to a behavior cage and recorded using a video camera for 1 h at fixed intervals of baseline, during MPTP injections, and in the post-MPTP period. The clinical rating was performed by two skilled and well-trained technicians according to the Kurlan rating scale [29]. In order to avoid subjective bias, the technicians were blinded to the study protocol. The rated symptoms/signs were facial expression (0–3), resting tremor (0–3), action or intention tremor (0–3), posture (0–2), gait (0–3), bradykinesia (generalized) (0–4), balance/coordination (0–3), gross motor skills for upper limb (0–3), gross motor skills for lower limb (0–3), and defense reaction (0–2). The minimum total score was 0, indicating normal behavior, and the maximum total score was 29, indicating severe disability.

Measurement of Overall Home-Cage Activity Level

The total home cage activity was assessed using a webcam monitoring system [30]. The animal was continuously monitored in the home cage for 8 h of the day from the beginning of MPTP injection. During levodopa treatment, the data were collected twice daily for 4 h each time after Madopar tube-feeding, for a total of 8 h of home cage activity.

Statistical Analysis

Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by a Bonferroni-corrected post hoc multiple comparison. Statistical significance was determined at the level of 0.05 for the P value. Data are expressed as mean ± SD. The statistical analysis was performed using SPSS 16.0 (SPSS Inc. Chicago, IL).

Results

Clinical Threshold Following Individualized MPTP Dosing Regimen

On days 10 to 13 (mean 11.60 ± 1.52) of MPTP administration, the 5 animals manifested typical PD symptoms of tremor, rigidity, and bradykinesia. The Kurlan scores were 10 to 11 (mean 10.40 ± 0.55), which represented the threshold of change from the asymptomatic to the symptomatic stage. The MPTP was stopped when the Kurlan score reached the threshold (Fig. 1).

Rapid Progress Stage (Post-MPTP Inertia)

After withdrawal of MPTP, the clinical symptoms of all 5 animals were exacerbated, with increases in the Kurlan scores. On days 3 to 12 (mean 6.40 ± 3.65) after MPTP withdrawal, the symptoms increased, representing the rapid

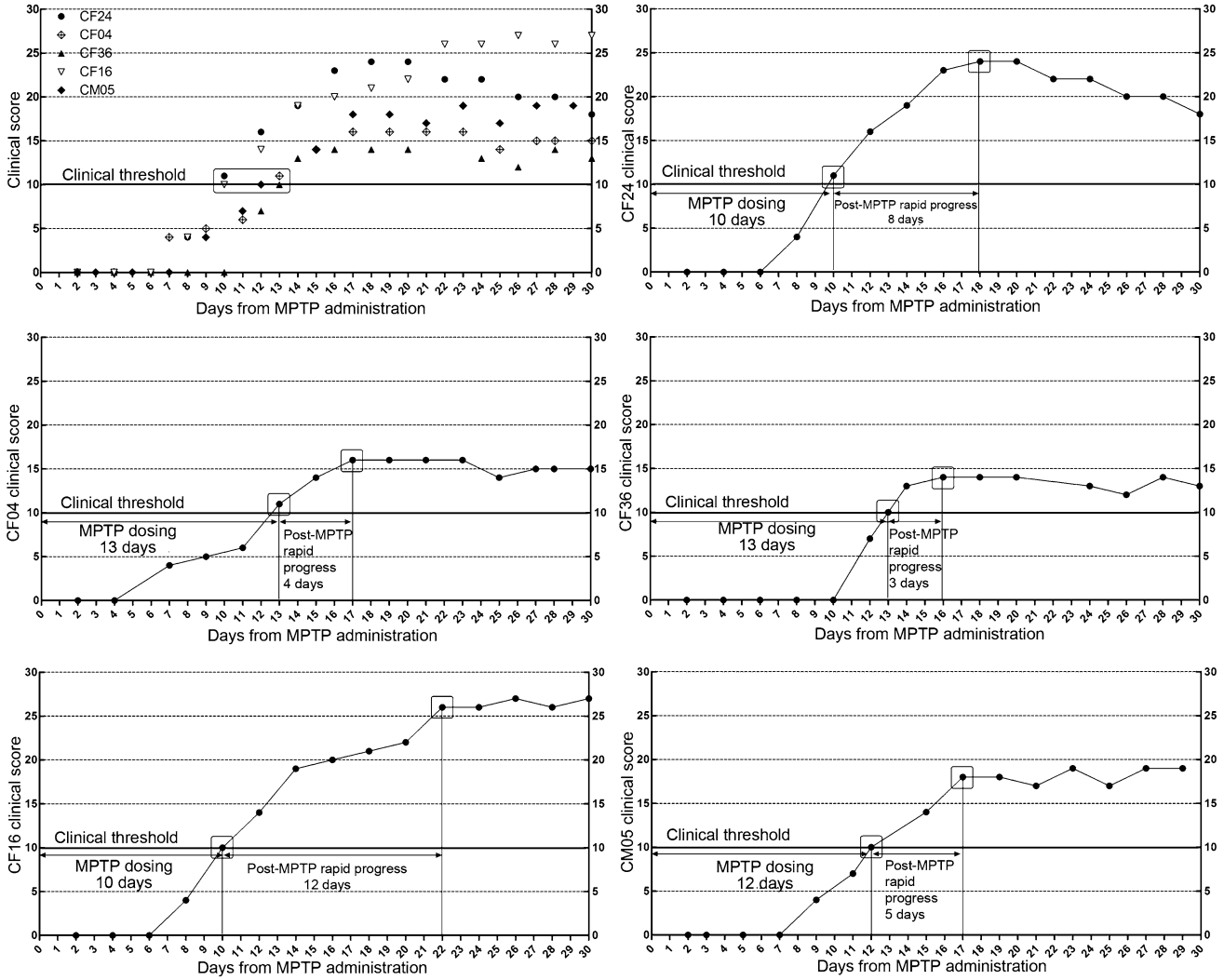

Fig. 1 Kurlan scores in 5 cynomolgus monkeys with MPTP administration during the pre-symptomatic and rapid progression stages.

progress stage (inertia effect), followed by a plateau. At the end of the rapid progress stage, the Kurlan scores reached the first peak at 24 (CF24), 16 (CF04), 14 (CF36), 26 (CF16), and 18 (CM05) days in the 5 animals (mean 19.60 ± 5.18) (Fig. 1).

Altered Clinical Evolution and Outcome and the Effect of Levodopa Treatment

The Kurlan scores and the total home-cage activity of the 5 monkeys during the 5 months following MPTP injection are shown in Figs. 2, 3, 4, 5, 6. After the rapid progress stage, the Kurlan scores of monkeys CF16, CF24, and CM05 remained stably high with slight fluctuations. The score of CF04 was also high and progressively increased, while that of CF36 increased in the two months after modeling and tended to decrease thereafter.

The monkeys were treated with levodopa at the end of the fifth month post-MPTP and showed different degrees of clinical improvement, reflected as reduced Kurlan scores and increased total amount of home cage activity (Figs. 2, 3, 4, 5, 6).

CF24

The Kurlan score increased to 24 during the rapid progress stage, and then declined to ∼18 on day 30. The score then slowly decreased to ∼15 by the end of month 4. After administration of levodopa, the score gradually decreased. Statistical analysis showed that the score in month 1 was higher than during the injection of MPTP ($P < 0.001$). It remained stable and decreased in month 5 without any significant difference from that in month 4. However, the

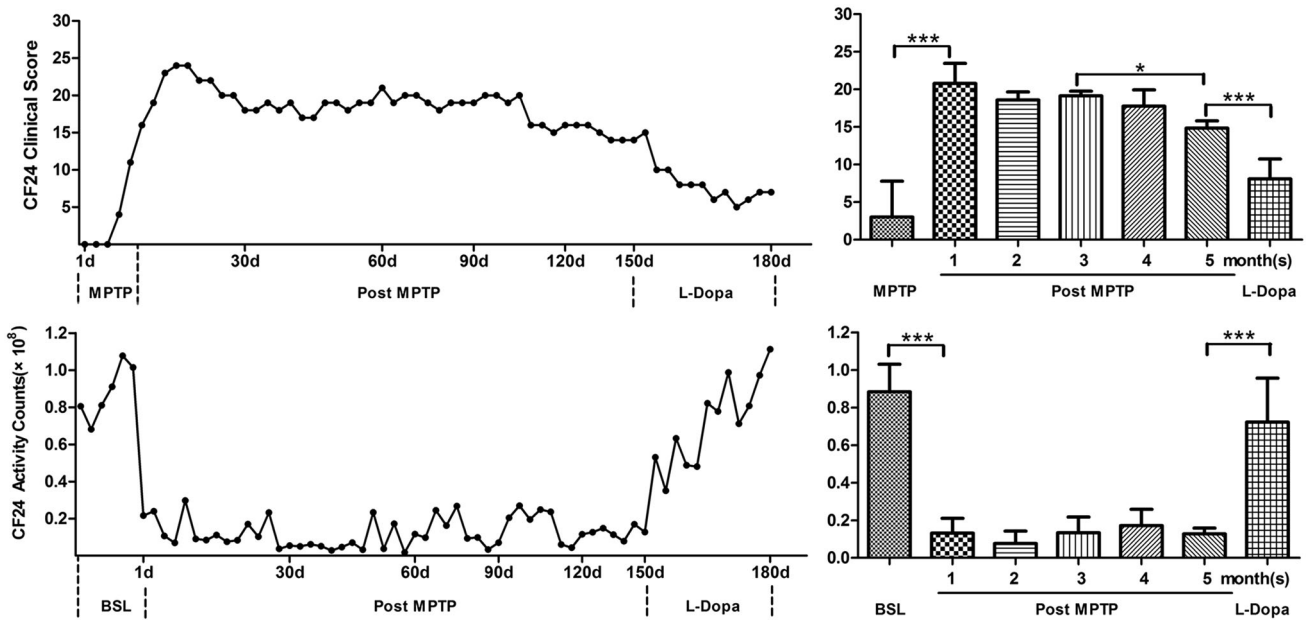

Fig. 2 Kurlan scores (upper panels) and total home-cage activity (lower panels) of CF24 during 5 months and after the administration of levodopa. BSL, baseline activity; MPTP, period of MPTP administration; L-Dopa, period of levodopa administration.

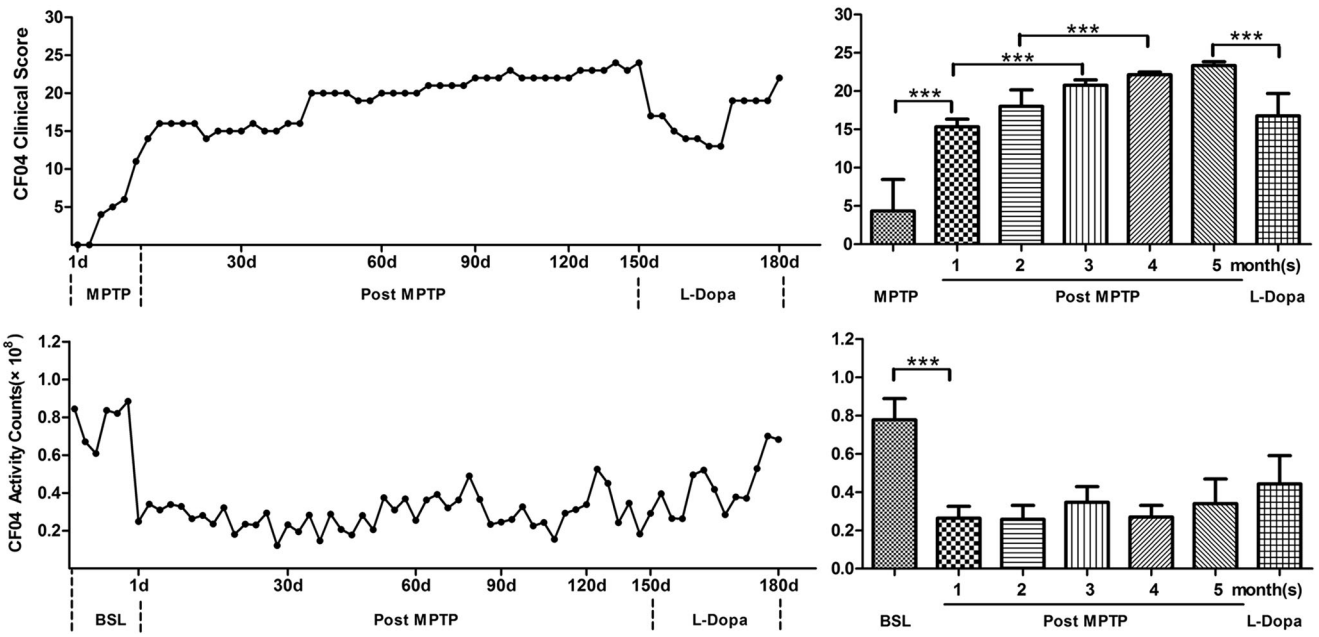

Fig. 3 Kurlan scores and total amount of home-cage activity of CF04 during 5 months and after levodopa administration. BSL, baseline activity; MPTP, period of MPTP administration; L-Dopa, period of levodopa administration.

score in month 5 was lower than in month 3 ($P < 0.05$). After the administration of levodopa, the score was lower than in month 5 ($P < 0.001$; Fig. 2).

The total activity quickly decreased after creation of the model and remained at a low level from month 1 to month 5, and gradually increased after administration of levodopa. Statistical analysis showed the activity in month 1 was lower than at baseline ($P < 0.001$). There was no difference between groups during months 1–5 ($P > 0.05$). The activity increased after the administration of levodopa when compared with that in month 5 ($P < 0.05$; Fig. 2).

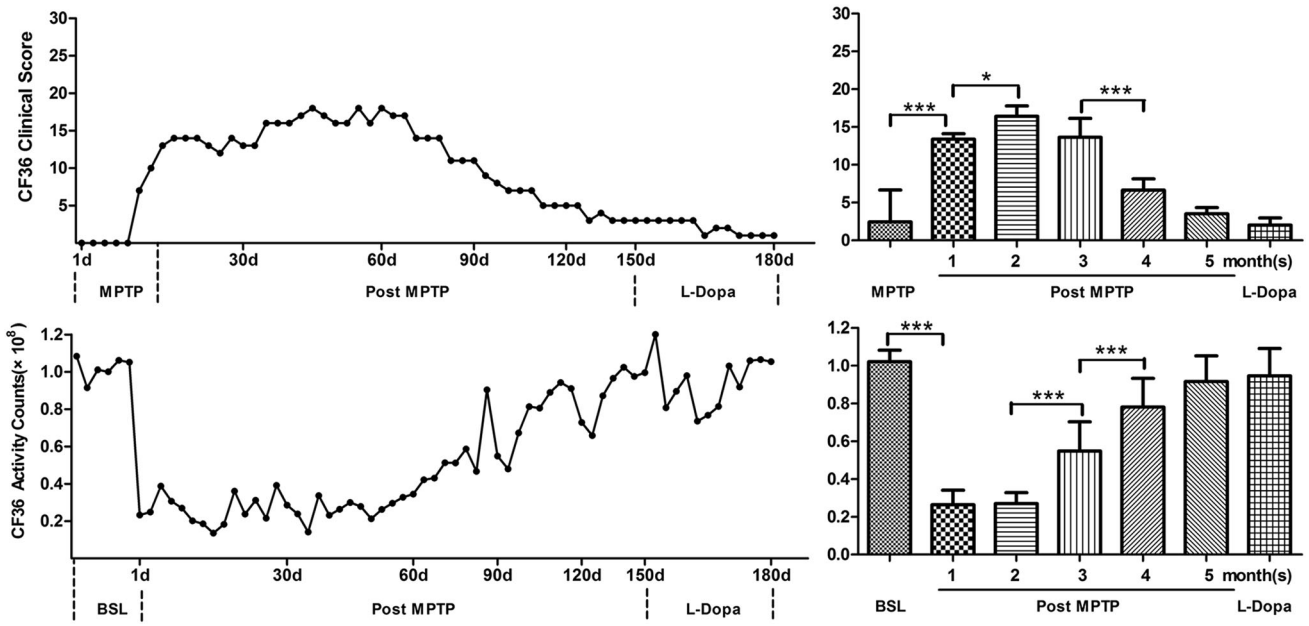

Fig. 4 Kurlan scores and total home-cage activity of CF36 during 5 months and after levodopa treatment. BSL, baseline activity; MPTP, period of MPTP administration; L-Dopa, period of levodopa administration.

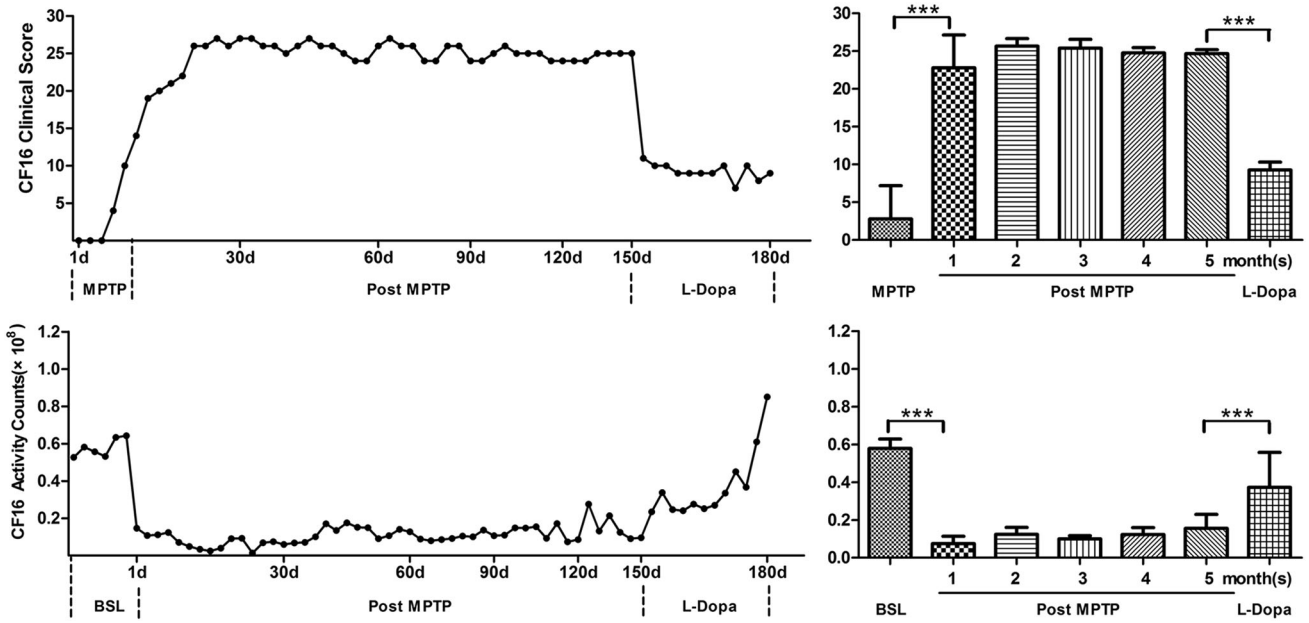

Fig. 5 Kurlan scores and total home-cage activity of CF16 during 5 months and after levodopa treatment. BSL, baseline activity; MPTP, period of MPTP administration; L-Dopa, period of levodopa administration.

CF04

The Kurlan score rapidly increased to 16 after injection of MPTP and persisted at this level until around day 45. The score tended to increase to $\sim$24 by day 150. Statistical analysis showed that the score increased in month 1 compared with the MPTP injection stage ($P < 0.001$). The score slowly increased from month 2 to month 5 without any significant difference from the previous month. The score in month 3 was higher than in month 1 ($P < 0.001$), and higher in month 4 than in month 2 ($P < 0.001$). After levodopa administration, the score decreased and then gradually increased. The level was lower than in month 5 ($P < 0.001$; Fig. 3).

The total activity quickly decreased after the model was established, remained low during the 5-month period, and

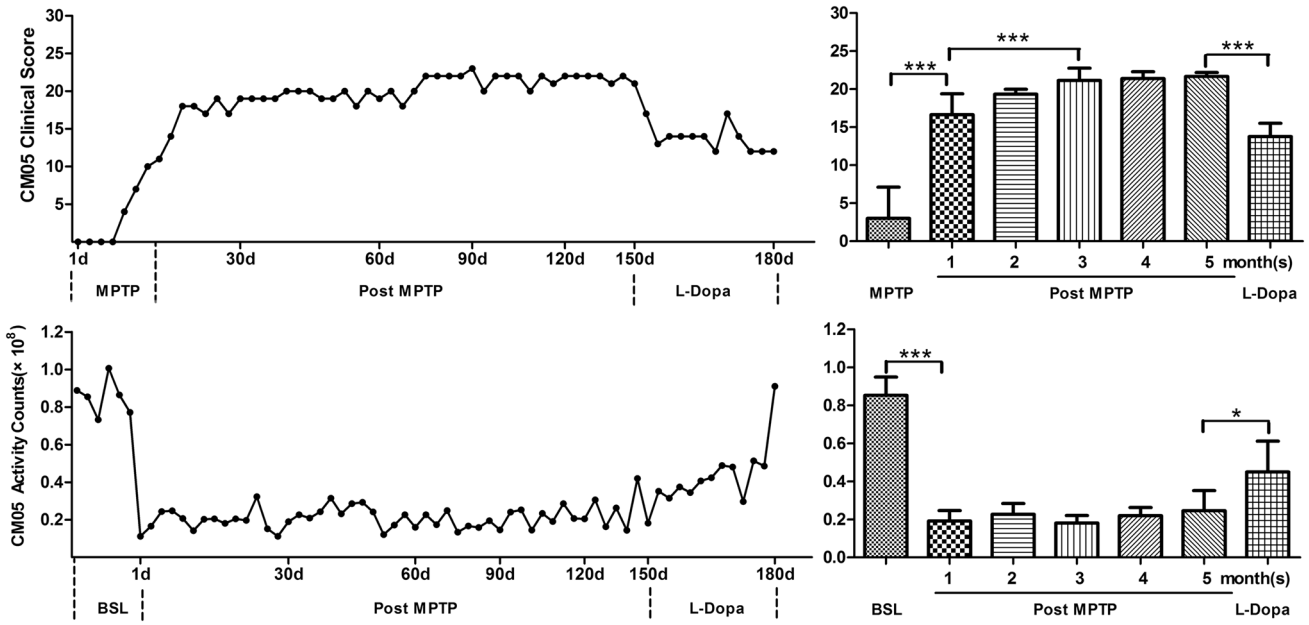

Fig. 6 Kurlan scores and total home-cage activity of CM05 during 5 months and after levodopa treatment. BSL, baseline activity; MPTP, period of MPTP administration; L-Dopa, period of levodopa administration.

gradually increased after levodopa administration without any significant change ($P > 0.5$). Statistical analysis showed that the activity in month 1 was less than at baseline ($P < 0.001$). There was no difference between groups during the five months ($P > 0.05$; Fig. 3).

CF36

The Kurlan score rapidly increased to ~14 after injection of MPTP. It then tended to increase and reached ~ 18 on day 70. It decreased to 10 on day 120 and 3 on day 150. After levodopa administration, the score declined to 0. Statistical analysis showed that the score increased in month 1 over the MPTP injection stage ($P < 0.001$). The score in month 2 was higher than in month 1 ($P < 0.05$). The score decreased from month 3, without any significant difference ($P = 0.059$) from month 2. The score in month 4 was lower than in month 3 ($P < 0.001$). The score in month 5 was lower than in month 4 without a significant difference. After levodopa administration, the score continued to decrease without any significant difference when compared with month 5 ($P > 0.05$; Fig. 4).

The total activity quickly decreased after the model was established and remained at a low level for 60 days, then gradually increased until day 150. Statistical analysis showed less activity in month 1 than at baseline ($P < 0.001$). There was no difference between the scores in months 1 and 2 ($P > 0.05$). The level in month 3 was higher than in month 2 ($P < 0.001$), higher in month 4 than in month 3 ($P < 0.001$), and higher in month 5 than in month 4, but without a statistical difference ($P > 0.05$). The activity continuously increased after the administration of levodopa relative to month 5 without any significant difference ($P > 0.05$; Fig. 4).

CF16

The Kurlan score rapidly increased to ~27 after injection of MPTP and remained at this level until the end of month 5. After levodopa treatment, the score decreased. Statistical analysis showed that the score increased in month 1 ($P < 0.001$). There was no significant difference between values from month 1 to month 5. After levodopa therapy, the score decreased compared with that in month 5 ($P < 0.001$; Fig. 5).

The total activity quickly decreased after creating the model and remained low until month 5. It gradually increased after levodopa administration. The activity in month 1 was less than at baseline ($P < 0.001$), with no significant difference between the scores in months 1 and 5 ($P > 0.05$). The activity increased after levodopa administration compared with that in month 5 ($P < 0.001$; Fig. 5).

CM05

The score rapidly increased to ~ 18 after injection of MPTP and persisted at this level until around day 70. The score then increased to 22 and remained at this level until

the end of month 5. After levodopa administration, the score decreased. Statistical analysis showed that the score increased in month 1 ($P < 0.001$), and gradually increased from month 1 to month 3, with no statistical difference from the previous month ($P > 0.05$). The score in month 3 was higher than in month 1 ($P < 0.001$). There was no difference between groups during months 3 to 5 ($P > 0.05$). After levodopa treatment, the score decreased compared with that in month 5 ($P < 0.001$; Fig. 6).

The total activity quickly decreased after the model was established and remained low until month 5. The activity gradually increased after levodopa administration. The activity in month 1 was less than at baseline ($P < 0.001$). The activity remained low from month 1 to month 5 with no significant difference between groups ($P > 0.05$). The activity increased after levodopa administration compared with that in month 5 ($P < 0.001$; Fig. 6).

Changes of Kurlan Score at the End of the Rapid Progress Stage and the End of Months 3 and 5

The Kurlan scores at the end of the rapid progress stage were 24 (CF24), 16 (CF04), 14 (CF36), 26 (CF16), and 18 (CM05). At the end of month 5, the score of C36 was <5, while the others all scored > 10 (Fig. 7).

Discussion

In this study, a systemic PD model using MPTP was induced at the individual level. A clinical rating scale (Kurlan score), which is significantly correlated with the severity of dopaminergic denervation [25, 54], was mainly used to evaluate the clinical evolution and outcomes. MPTP was stopped when each animal manifested symptoms typical of clinical PD at different times (mean 11.6 ± 1.52 days), as the Kurlan score increased to ~10 (mean 10.4 ± 0.55). Therefore, we believe that a Kurlan score of 10 is the threshold between the preclinical asymptomatic and the clinical symptomatic stage, similar to the progression of primary PD. The death of substantia nigra neurons and subsequent clinical symptoms is attributed to age-related degeneration and lesions in the substantia nigra pars compacta [31]. Clinical symptoms appear when 50%–60% of dopaminergic neurons and >70%–80% of striatal nerve terminals have degenerated or died. The concept of a clinical symptom threshold is based on data derived from decreased dopamine levels in post-mortem tissue from humans and altered dopaminergic function observed in radioisotopic brain imaging [26, 32, 33].

Before the appearance of symptoms, the numbers of TH-positive cells in the substantia nigra are reduced. However, most cells are still intact, so the symptoms are attributed to the lesions of functional integrity in the dopaminergic terminals of the substantia nigra [14, 53].

Our study showed that the symptoms in 5 animals were exacerbated and peaked 3–12 days (mean 6.40 ± 3.65) after discontinuing MPTP, when the Kurlan score reached a first peak at 14–26 points (mean 19.60 ± 5.18). This period was termed the post-MPTP rapid progress or inertia stage.

In primary PD, the degeneration of dopaminergic neurons accelerates from the onset of symptoms to a stable stage, at which it is suggested that the cells have been destroyed. The mechanism underlying this accelerated degeneration is triggered upon reaching the threshold of symptom onset [14]. The changes from the onset of PD symptoms to the rapid progress stage in this study simulated the early phase of primary PD. The Kurlan scores of monkeys rapidly peaked at different times (3–12 days) after MPTP discontinuation, suggesting individual differences in the degeneration of dopaminergic neurons in the substantia nigra when the MPTP lesions reach the threshold. This result is partly attributable to individual differences in sensitivity to MPTP. However, it provides a therapeutic window for early intervention in PD.

During the transition from the rapid progress stage to month 5, the clinical evolution and outcomes of the 5 animals were dynamically evaluated. Only one animal showed spontaneous recovery, while the remaining four manifested sustained clinical PD, three in a stable condition and one remaining in a progressive state. After levodopa treatment, the four animals showed different degrees of behavioral improvement. The clinical status in the animal with spontaneous recovery was almost normal before levodopa administration.

Many previous studies have reported spontaneous recovery in the non-human primate model of PD, suggesting partial or complete behavioral recovery over time in some animals. The possible factors in spontaneous recovery might be related to the species, the age of the animal, and the method of MPTP application [15, 19–21, 29, 34–38]. Studies have indicated that the level of initial dopamine depletion induced by MPTP may be the most critical factor in spontaneous recovery, indicating that the initial severity of the deficit is an important predictor of outcome [20, 39]. The clinical severity corresponding to the Kurlan score at the end of the rapid progress stage in this study may represent the initial level of damage to the dopaminergic system induced by MPTP. The score of the monkey manifesting spontaneous recovery was 14 at the end of rapid progress stage, much lower than that of the other animals (16–26). Studies have suggested that stable Parkinsonism occurs in severe cases despite varying degrees of response to MPTP treatment [20]. The occurrence of spontaneous recovery and stable PD in

Animal	CRS at end of rapid progess	CRS of 3rd month	CRS of 5th month
CF24	24	19.13±0.64	14.83±0.98
CF04	16	20.75±0.71	23.33±0.52
CF36	14	13.63±2.50	3.50±0.84
CF16	26	25.38±1.19	24.67±0.52
CM05	18	21.13±1.64	21.67±0.52

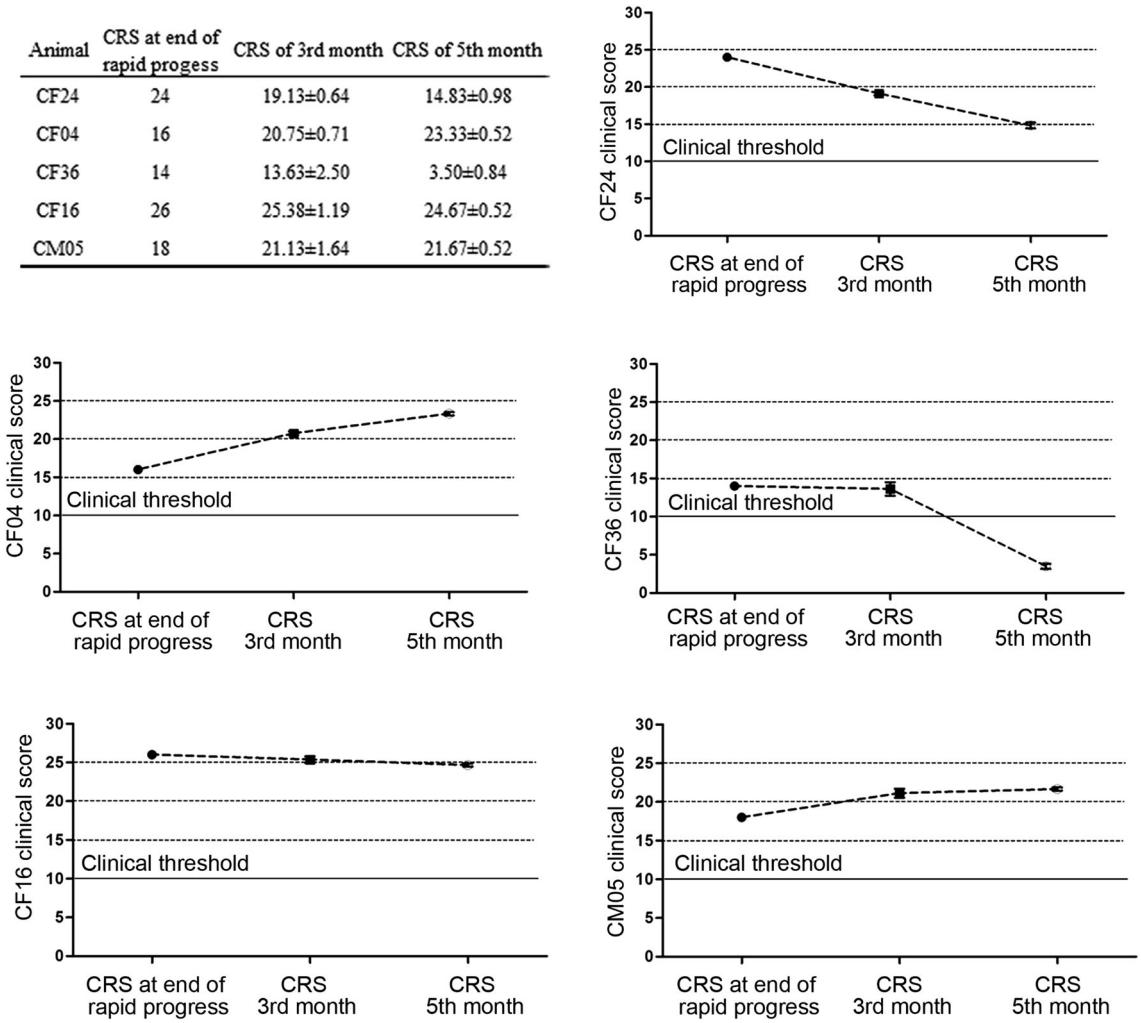

Fig. 7 Kurlan scores at the end of the rapid progress stage and the end of months 3 and 5.

monkeys also suggests that milder initial lesions of dopaminergic neurons are associated with a greater capacity for regeneration or sprouting of dopamine terminals [36].

A score >15 at the end of the rapid progress stage suggested a decreased chance of spontaneous recovery and reflected a stable clinical model of PD. This may be regarded as a good predictor of clinical outcome, especially for a spontaneous recovery PD model.

The age of the non-human primate is one of the most important factors for a successful MPTP PD model. Based on the PD model of Bezard [14, 25, 27, 40], we selected middle-aged cynomolgus monkeys for this study. Primary PD mainly occurs in the elderly, and old age is an important factor underlying the incidence of PD. In human and non-human primates, the dopaminergic neurons in the substantia nigra degenerate with advanced age [41–48], especially when exposed to pathogenic factors [19, 24]. The plasticity of the substantia nigra in the aged animal is relatively low, and this decreases the probability of spontaneous recovery in the PD model. Studies of primary PD suggest that, compared with elderly patients, younger patients develop more slowly and endure long-term damage to the dopaminergic system before manifesting the first motor symptoms [49]. These reports suggest that younger PD patients have more efficient compensatory mechanisms. Studies have demonstrated that aging in rhesus monkeys is three times faster than in humans [50]. The average life-span of a cynomolgus monkey is ~25 years, and the maximum recorded was 37 years [51]. In contrast, the maximum life-span in humans is 120 years [52]. Therefore, in the cynomolgus monkey, 1 year is equivalent to 4 years of human life, so the animals in this study were middle-aged or elderly.

The individualized intravenous injections with small-dose MPTP in middle-aged cynomolgus monkeys led to the appearance of typical clinical symptoms on days 10 to 13, (mean 11.60 ± 1.52) earlier than in the 3-year-old monkey

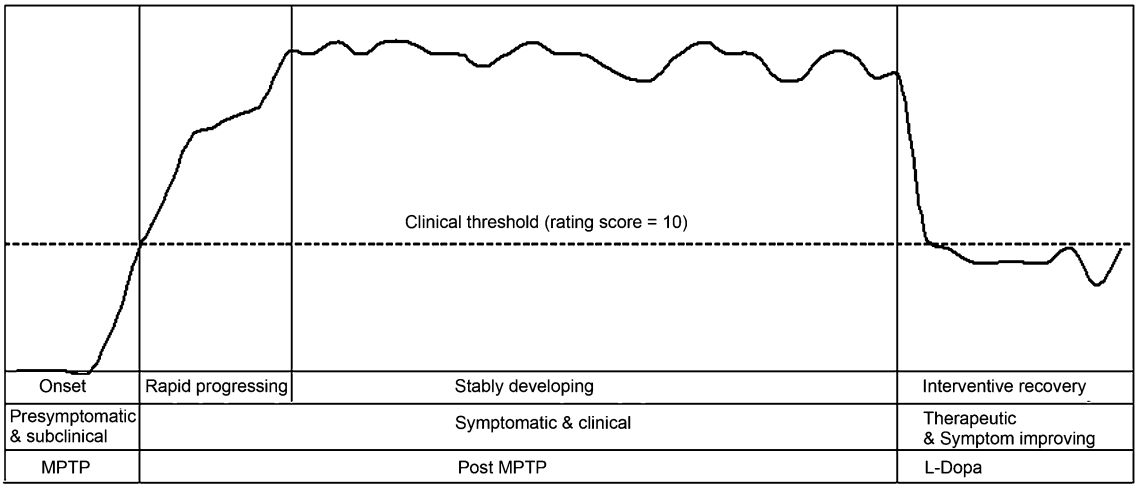

Fig. 8 Typical clinical evolution and outcomes in a systemic PD MPTP model using middle-aged cynomolgus monkeys.

(15 ± 1 days) under similar conditions [14, 53]. This suggests that the sensitivity to MPTP increases with age [19]. It also suggests greater individual differences in sensitivity to MPTP in middle-aged monkeys than in their younger counterparts. Therefore, the induction of PD models by individualized MPTP abrogates the individual differences in susceptibility to MPTP and reduces the occurrence of spontaneous recovery.

One point needs to be explained; since ketamine was used in this study, and it is known to be an N-methyl-D-aspartate antagonist, it may have had a protective effect on the MPTP-induced neurotoxic lesion [55, 56] and hence might have slightly ameliorated the evolution of parkinsonian symptoms. But a systemic influence was avoided by using the same protocol in each monkey and so did not influence the overall profile of the results.

In conclusion, the systemic PD model we established in middle-aged cynomolgus monkeys with MPTP reduced the differences in individual susceptibility. MPTP was stopped upon reaching the clinical threshold of PD. The clinical score at the end of the rapid progress stage was a good prognostic indicator of clinical evolution and outcome, as well as the possibility of spontaneous recovery. During the 5-month follow-up, the Kurlan score of the animal with spontaneous recovery decreased during months 2 and 3, to <15 at the end of month 3, and was almost 0 by the end of month 5. The Kurlan scores of the four animals in stable and progressive status were >15 at the end of the rapid progress stage and ~ 20 and above at the end of month 3. Those animals were still in the clinical state of PD at the end of month 5. Therefore, a Kurlan score >15 at the end of the rapid progress stage and the end of month 3 indicates a diminished possibility of spontaneous recovery. Continuous PD symptoms for at least 3 months represent a stable and effective PD model. The process of clinical evolution and the outcome of systemic PD in a cynomolgus monkey model were well characterized in this study (Fig. 8) to serve as a basis for additional investigations.

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References

1. Collier TJ, Steece-Collier K, Kordower JH. Primate models of Parkinson's disease. Exp Neurol 2003, 183: 258–262.
2. Emborg ME. Evaluation of animal models of Parkinson's disease for neuroprotective strategies. J Neurosci Methods 2004, 139: 121–143.
3. Emborg ME. Nonhuman primate models of Parkinson's disease. ILAR J 2007, 48: 339–355.
4. Capitanio JP, Emborg ME. Contributions of non-human primates to neuroscience research. Lancet 2008, 371: 1126–1135.
5. Jenner P. From the MPTP-treated primate to the treatment of motor complications in Parkinson's disease. Parkinsonism Relat Disord 2009, 15 Suppl 4: S18–23.
6. Blesa J, Phani S, Jackson-Lewis V, Przedborski S. Classic and new animal models of Parkinson's disease. J Biomed Biotechnol 2012, 2012: 845618.
7. Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci U S A 1983, 80: 4546–4550.
8. Chiueh CC, Markey SP, Burns RS, Johannessen JN, Jacobowitz DM, Kopin IJ. Neurochemical and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig, and monkey. Psychopharmacol Bull 1984, 20: 548–553.

9. Langston JW, Forno LS, Rebert CS, Irwin I. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. Brain Res 1984, 292: 390–394.
10. Doudet D, Gross C, Lebrun-Grandie P, Bioulac B. MPTP primate model of Parkinson's disease: a mechanographic and electromyographic study. Brain Res 1985, 335: 194–199.
11. Schultz W, Studer A, Jonsson G, Sundstrom E, Mefford I. Deficits in behavioral initiation and execution processes in monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism. Neurosci Lett 1985, 59: 225–232.
12. Bankiewicz KS, Oldfield EH, Chiueh CC, Doppman JL, Jacobowitz DM, Kopin IJ. Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Life Sci 1986, 39: 7–16.
13. Benazzouz A, Gross C, Dupont J, Bioulac B. MPTP induced hemiparkinsonism in monkeys: behavioral, mechanographic, electromyographic and immunohistochemical studies. Exp Brain Res 1992, 90: 116–120.
14. Bezard E, Dovero S, Prunier C, Ravenscroft P, Chalon S, Guilloteau D, *et al.* Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. J Neurosci 2001, 21: 6853–6861.
15. Eidelberg E, Brooks BA, Morgan WW, Walden JG, Kokemoor RH. Variability and functional recovery in the N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of parkinsonism in monkeys. Neuroscience 1986, 18: 817–822.
16. Jenner P, Marsden CD. The actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in animals as a model of Parkinson's disease. J Neural Transm Suppl 1986, 20: 11–39.
17. Elsworth JD, Deutch AY, Redmond DE, Jr., Taylor JR, Sladek JR, Jr., Roth RH. Symptomatic and asymptomatic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates: biochemical changes in striatal regions. Neuroscience 1989, 33: 323–331.
18. Kurlan R, Kim MH, Gash DM. The time course and magnitude of spontaneous recovery of parkinsonism produced by intracarotid administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to monkeys. Ann Neurol 1991, 29: 677–679.
19. Ovadia A, Zhang Z, Gash DM. Increased susceptibility to MPTP toxicity in middle-aged rhesus monkeys. Neurobiol Aging 1995, 16: 931–937.
20. Taylor JR, Elsworth JD, Roth RH, Sladek JR, Jr., Redmond DE, Jr. Severe long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the vervet monkey (Cercopithecus aethiops sabaeus). Neuroscience 1997, 81: 745–755.
21. Boulet S, Mounayar S, Poupard A, Bertrand A, Jan C, Pessiglione M, *et al.* Behavioral recovery in MPTP-treated monkeys: neurochemical mechanisms studied by intrastriatal microdialysis. J Neurosci 2008, 28: 9575–9584.
22. Ding F, Luan L, Ai Y, Walton A, Gerhardt GA, Gash DM, *et al.* Development of a stable, early stage unilateral model of Parkinson's disease in middle-aged rhesus monkeys. Exp Neurol 2008, 212: 431–439.
23. Fox SH, Brotchie JM. The MPTP-lesioned non-human primate models of Parkinson's disease. Past, present, and future. Prog Brain Res 2010, 184: 133–157.
24. Morrow BA, Roth RH, Redmond DE, Jr., Diano S, Elsworth JD. Susceptibility to a parkinsonian toxin varies during primate development. Exp Neurol 2012, 235: 273–281.
25. Bezard E, Imbert C, Deloire X, Bioulac B, Gross C. A chronic MPTP model reproducing the slow evolution of Parkinson's disease: evolution of motor symptoms in the monkey. Brain Res 1997, 766: 107–112.
26. Marek K, Jennings D, Tamagnan G, Seibyl J. Biomarkers for Parkinson's [corrected] disease: tools to assess Parkinson's disease onset and progression. Ann Neurol 2008, 64 Suppl 2: S111–121.
27. Bezard E, Imbert C, Deloire X, Bioulac B, Gross CE. A chronic MPTP model reproducing the slow evolution of Parkinson's disease: evolution of motor symptoms in the monkey. Brain Res 1997, 766: 107–112.
28. Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, *et al.* The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem 2001, 76: 1265–1274.
29. Kurlan R, Kim MH, Gash DM. Oral levodopa dose-response study in MPTP-induced hemiparkinsonian monkeys: assessment with a new rating scale for monkey parkinsonism. Mov Disord 1991, 6: 111–118.
30. Yue F, Zeng S, Wu D, Yi D, Alex Zhang Y, Chan P. Age-related decline in motor behavior and striatal dopamine transporter in cynomolgus monkeys. J Neural Transm 2012, 119: 943–952.
31. Koller WC, Langston JW, Hubble JP, Irwin I, Zack M, Golbe L, *et al.* Does a long preclinical period occur in Parkinson's disease? Neurology 1991, 41: 8–13.
32. Hornykiewicz O, Kish SJ. Biochemical pathophysiology of Parkinson's disease. Adv Neurol 1987, 45: 19–34.
33. Morrish PK, Sawle GV, Brooks DJ. An [18F]dopa-PET and clinical study of the rate of progression in Parkinson's disease. Brain 1996, 119 (Pt 2): 585–591.
34. Perez-Otano I, Herrero MT, Luquin MR, Obeso JA, Del Rio J. Chronic MPTP treatment reduces substance P and met-enkephalin content in the basal ganglia of the marmoset. Brain Res 1992, 585: 156–160.
35. Gaspar P, Febvret A, Colombo J. Serotonergic sprouting in primate MTP-induced hemiparkinsonism. Exp Brain Res 1993, 96: 100–106.
36. Elsworth JD, Taylor JR, Sladek JR, Jr., Collier TJ, Redmond DE, Jr., Roth RH. Striatal dopaminergic correlates of stable parkinsonism and degree of recovery in old-world primates one year after MPTP treatment. Neuroscience 2000, 95: 399–408.
37. Song DD, Haber SN. Striatal responses to partial dopaminergic lesion: evidence for compensatory sprouting. J Neurosci 2000, 20: 5102–5114.
38. Mounayar S, Boulet S, Tande D, Jan C, Pessiglione M, Hirsch EC, *et al.* A new model to study compensatory mechanisms in MPTP-treated monkeys exhibiting recovery. Brain 2007, 130: 2898–2914.
39. Waters CM, Hunt SP, Jenner P, Marsden CD. An immunohistochemical study of the acute and long-term effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the marmoset. Neuroscience 1987, 23: 1025–1039.
40. Bezard E, Boraud T, Bioulac B, Gross CE. Compensatory effects of glutamatergic inputs to the substantia nigra pars compacta in experimental parkinsonism. Neuroscience 1997, 81: 399–404.
41. McGeer PL, McGeer EG, Suzuki JS. Aging and extrapyramidal function. Arch Neurol 1977, 34: 33–35.
42. Wenk GL, Pierce DJ, Struble RG, Price DL, Cork LC. Age-related changes in multiple neurotransmitter systems in the monkey brain. Neurobiol Aging 1989, 10: 11–19.
43. Felten DL, Felten SY, Steece-Collier K, Date I, Clemens JA. Age-related decline in the dopaminergic nigrostriatal system: the oxidative hypothesis and protective strategies. Ann Neurol 1992, 32 Suppl: S133–136.
44. Irwin I, DeLanney LE, McNeill T, Chan P, Forno LS, Murphy GM, Jr., *et al.* Aging and the nigrostriatal dopamine system: a non-human primate study. Neurodegeneration 1994, 3: 251–265.
45. Gerhardt GA, Cass WA, Henson M, Zhang Z, Ovadia A, Hoffer BJ, *et al.* Age-related changes in potassium-evoked overflow of dopamine in the striatum of the rhesus monkey. Neurobiol Aging 1995, 16: 939–946.

46. Emborg ME, Ma SY, Mufson EJ, Levey AI, Taylor MD, Brown WD, *et al.* Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. J Comp Neurol 1998, 401: 253–265.
47. Kanaan NM, Kordower JH, Collier TJ. Age-related changes in dopamine transporters and accumulation of 3-nitrotyrosine in rhesus monkey midbrain dopamine neurons: relevance in selective neuronal vulnerability to degeneration. Eur J Neurosci 2008, 27: 3205–3215.
48. Hurley P, Elsworth J, Whittaker M, Roth R, Redmond D. Aged monkeys as a partial model for Parkinson's disease. Pharmacology Biochemistry and Behavior 2011, 99: 324–332.
49. de la Fuente-Fernandez R, Schulzer M, Kuramoto L, Cragg J, Ramachandiran N, Au WL, *et al.* Age-specific progression of nigrostriatal dysfunction in Parkinson's disease. Ann Neurol 2011, 69: 803–810.
50. Andersen AH, Zhang Z, Zhang M, Gash DM, Avison MJ. Age-associated changes in rhesus CNS composition identified by MRI. Brain Res 1999, 829: 90–98.
51. Jones ML. Longevity of captive mammals. Zoologische Garten 1982, 52: 113-128.
52. Robine JM, Allard M. The oldest human. Science 1998, 279: 1834–1835.
53. Meissner W, Prunier C, Guilloteau D, Chalon S, Gross CE, Bezard E. Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. Mol Neurobiol 2003, 28: 209–218.
54. Liu Y, Yue F, Tang R, Tao G, Pan X, Zhu L, *et al.* Progressive loss of striatal dopamine terminals in MPTP-induced acute parkinsonism in cynomolgus monkeys using vesicular monoamine transporter type 2 PET imaging ([(18)F]AV-133). Neurosci Bull 2014, 30: 409–416.
55. Araki T1, Kumagai T, Tanaka K, Matsubara M, Kato H, Itoyama Y, *et al.* Neuroprotective effect of riluzole in MPTP-treated mice. Brain Res. 2001, 918: 176–181.
56. Ferro MM1, Angelucci ME, Anselmo-Franci JA, Canteras NS, Da Cunha C. Neuroprotective effect of ketamine/xylazine on two rat models of Parkinson's disease. Braz J Med Biol Res 2007, 40: 89–96.