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Non-human primate FOG develops with advanced parkinsonism induced by MPTP Treatment

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Abstract

Freezing of gait (FOG) is a debilitating feature of Parkinson's disease (PD) and other forms of parkinsonism. The anatomical or pathophysiological correlates are poorly understood largely due to the lack of a well-established animal model. Here we studied whether FOG is reproduced in the non-human primate (NHP) model of PD. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Genus *Macaca*, n=29) were examined for the development of FOG, and the leg movements were recorded with accelerometry. The relationships between developing FOG and the animals' characteristics, the MPTP treatments, and the modeled outcomes were determined. In parkinsonian monkeys FOG developed frequently (48%) manifesting similar characteristics to those seen in PD patients. In addition, FOG episodes in the monkey were accompanied by leg trembling with the typical duration (2–10 s) and frequency (~7 Hz). The development of *NHP FOG* was significantly associated with the severity of parkinsonism, as shown by high motor disability scores (> 20) and levodopa-induced dyskinesia scores ($p=0.01$ and $p=0.04$, respectively). Differences in demographics and MPTP treatments (doses, treatment duration, etc.) had no influence on *NHP FOG* occurrence, with the exception of gender that showed FOG predominance in males ($p=0.03$). The unique features of FOG in PD can be replicated in severely parkinsonian macaques, and this represents the first description of a FOG animal model.

Keywords

Freezing of gait; MPTP; Animal model; Non-human primate; Dopamine

Introduction

Freezing of gait (FOG) is a poorly understood, disabling disorder that develops in Parkinson's disease (PD) (Giladi et al., 1992) and other parkinsonian syndromes (Factor, 2008; Nutt et al., 2011). It is defined as an episodic inability to generate effective stepping in the absence of any known cause other than parkinsonism or high level gait disorders (Fahn, 1995; Giladi et al., 1992; Giladi and Nieuwboer, 2008). In PD, FOG occurs in various

situations, most commonly with initiating gait and with turning (Fahn, 1995; Giladi, et al., 1992). Types of FOG include: those with shuffling gait movements without forward motion (the slipping clutch), leg trembling but feet stuck to the floor, and complete akinesia (no movement at all) (Schaafsma et al., 2003; Thompson and Marsden, 1995). Initially, once the freezing is broken, patients can walk almost normally, but as the disease progresses, FOG is often accompanied by gait festination, postural instability and falling, ultimately requiring a wheelchair (Grimbergen et al., 2004). In PD, FOG typically occurs in the “off” state, but has also been described in the “on” state. The “off”-state FOG may respond to levodopa (Schaafsma et al., 2003); however, resistance to dopaminergic drug treatment has been observed frequently leading to an “on”-state FOG (Factor, 2001; Holloway et al., 2004; Rascol et al., 2000). This disabling symptom is a significant cause of falls, interferes with activities of daily living, causes social isolation and has a negative effect on overall quality of life (Bloem et al., 2004; Gurevich et al., 2007; Macht et al., 2007).

The prominent occurrence of FOG in association with PD and the relation to the “off” state in PD has led to its link to the hypodopaminergic state. However, the appearance or persistence of FOG in the “on” state, and even together with levodopa-induced dyskinesias (Giladi et al., 1992), has suggested that non-dopaminergic mechanisms may also play a role. Notably, FOG occurring in later stages of disease is generally more severe, and tends to occur more in both “off” and “on” states, likely suggesting the appearance of treatment resistance as disease progresses. The development of this symptom in PD has been related to changes in various circuits including the pedunculopontine nucleus (Devos et al., 2010) and frontal cortex-basal ganglia connections (Amboni et al., 2010; Bartels and Leenders, 2008; Factor, 2008), which involve the participation of multiple neurotransmitter systems (Devos et al., 2010). However, the anatomical and biochemical correlates of FOG remain unclear. As a result, pharmacological therapies manipulating the dopaminergic or other systems have failed thus far (Giladi and Nieuwboer, 2008), and surgical therapies using deep brain stimulation of the subthalamic nucleus and PPN are currently under investigation (Ferraye et al., 2008; Moreau et al., 2009; Pereira et al., 2008). One important limitation in the research for this disorder has been the lack of an animal model. While freezing of movement in any part of the body, as a form of extreme akinesia, and various postural and gait abnormalities have been reported in parkinsonian monkeys (Jenner, 2003; Karachi et al., 2010), FOG, specifically, has not been reproduced in established rodent and primate models. The purpose of this study was to examine whether FOG can be replicated in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) non-human primate (NHP) model of PD.

Here, we report the development of *NHP FOG* in parkinsonian macaques, exhibiting a similar phenotype of the gait disturbance found in PD patients. The denomination of *NHP FOG* is intended to highlight species related differences in these episodes between non-human primates and humans. Since different degrees of parkinsonism can be achieved in these monkeys, we tested the hypothesis that the development of FOG might be dependent on the severity of parkinsonism. Our findings support this notion making this is the first description of reproducible *NHP FOG* with relative high frequency in markedly disabled, “advanced” MPTP parkinsonian monkeys. With few exceptions (Forno et al., 1986), MPTP has been reported to be toxic selectively to dopaminergic neurons in non-human primates (Jenner, 2003; Schmidt and Ferger, 2001), but such lesion results in a number of network abnormalities and distant secondary changes. These facts along with findings of *NHP FOG* development with MPTP lesions could provide insight into the pathophysiology of FOG in PD.

Materials and methods

Primate MPTP model

Data were collected by prospective examination of 6 monkeys, and retrospective review of clinical records of 23 monkeys that were used in other studies. Thus, a total of 29 macaques were included. Videos were reviewed when available. *Macaca mulatta* (n=20) and *Macaca fascicularis* (n=9), were males (n=12) and females (n=17) of adult age (2 to 10 years old). The average age ($\pm$ STD) was 4.3 ± 2.2 years (age records were missing in 3 monkeys). The average weight was 5.1 ± 2.1 kg. All had previously received MPTP systemically (i.v.) according to our standard methods (Cao et al., 2007; Papa and Chase, 1996). Briefly, the toxin was administered at weekly or longer intervals with a dose range of 0.5–1.5 mg/kg, i.v. until stable parkinsonism developed (usually after several months). Monkeys developed a chronic parkinsonism from mild to severe degree, and some were maintained on oral levodopa treatment (a few animals started antiparkinsonian treatment with the D₁ agonist SKF82958, and after developing dyskinesias their treatment continued with levodopa). All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and with approval by the Institutional Animal Care and Use Committee.

Behavioral data

For the purpose of this study *NHP FOG* was defined as an episodic inability to generate effective stepping during walking. Detailed documentation of the episode duration, and the presence or absence of leg trembling was available in the majority of charts reviewed. In a few charts, there was only a mention of *NHP FOG*, without a detailed description. The absence of *NHP FOG* was not noted in the records. Five videotaped evaluations that were available from the retrospective study were also reviewed, of which three showed *NHP FOG*. Six monkeys were evaluated prospectively. In order to determine if they had FOG (according to the applied definition) each monkey was observed for several days in the mornings prior to receiving their levodopa treatment. When FOG was identified (n=2), these monkeys were videotaped for 1–2 h for several days. It is important to note that the monkeys were stimulated and offered cues (food treat offers) to motivate their ambulation during examinations, and ensure alertness at the time of evaluation. Following direct examination of monkeys or review of the clinical records to determine the presence of *NHP FOG*, the following data were collected: demographics (species, gender, age, and weight), MPTP lesion (range of single doses, total number of injections, total cumulative dose [TCD], total duration of treatment), and behavioral outcomes of the MPTP model. These outcomes included: total motor disability scores (MDS) in the “off” state (motor disability scale for MPTP-treated primates; scale range: 0–39) and the maximal total dyskinesia scores (TDS; Part II of the motor disability scale) (Cao et al., 2007; Liang et al., 2008; Papa and Chase, 1996) obtained at the peak “on” state following levodopa methyl ester plus benserazide given subcutaneously at standard doses (125/31.25 mg). *NHP FOG* developed during levodopa (Sinemet 25/100) or SKF82958 (a selective dopamine D₁ agonist given s.c.) treatment. Total daily doses of this treatment were also documented for all animals. Clinical characteristics of FOG were documented. Videotaped examinations of two monkeys accompany this manuscript.

Tremor analysis

Direct examination of *NHP FOG* often revealed the presence of tremulousness of the legs. Attempts to record these shaking movements with a standard uniaxial accelerometer placed on the back of the hind limb frequently failed in one of the two monkeys in the prospective study (the animal detached the device from the leg quickly after being released to walk freely in the cage). However, the recordings could occasionally be maintained, and sufficient

accelerometry data (20 freezing episodes) were obtained for assessment of the tremor characteristics in the second monkey. The sensitive axis of the accelerometer was oriented parallel to the movement direction, and the signals sampled at 20 KHz. Data were analyzed with Plexon and NeuroExplorer systems. The criteria for determining the tremor episode were, 1) oscillatory activity exceeding a threshold set at the second phase of the curve in the “peak heights histogram”, and this threshold was usually above 2 standard deviations from the mean (background noise), 2) the presence of 4 or more full phase oscillations, and 3) the beginning and end of full phase oscillations. The tremor rate (Hz) and duration were calculated.

Statistical analysis

Fisher’s exact tests (for categorical variables) and Wilcoxon rank sum tests (for continuous variables) were used to compare the associations between monkeys that developed FOG and those that did not. Since we hypothesized that *NHP FOG* developed with significantly higher MDS and dyskinesia scores, one-sided hypothesis testing was used for these two tests. Two-sided hypothesis testing was used for all other comparisons.

Results

Characteristics of FOG in MPTP-treated macaques

NHP FOG developed in 14 out of 29 monkeys (48%) with stable parkinsonism, as follows: 2 monkeys were from the prospective examination group of 6 animals, and 12 from the retrospective review group of 23 animals (videotapes of *NHP FOG* were available on 5 cases in retrospective review). *NHP FOG* were sudden episodes lasting from several seconds to approximately 1 min, with clear evidence that the monkeys were able to walk without freezing before and after each episode (see videotapes in Supplementary materials). The frequency of *NHP FOG* was not documented, however, it was consistently observed in successive morning “off” state evaluations. We could not identify any specific provoking factors, although typical provoking factors for humans were not utilized due to the inherent difficulties of evaluating caged monkeys. In some cases there was evidence of hesitation or trembling of the hind limb. In addition, when the monkey’s gait froze, there appeared to be an associated generalized akinesia. *NHP FOG* could be observed during walking and climbing. A clear relation to starting or reaching the destination could not be established because of our inability to determine the animal’s intention to stop suddenly or his predetermined destination. Monkeys with FOG also had a tendency to slowly flex the legs until sitting down after they froze, which does not occur in humans. But in the monkey, sitting is an easily reached position from the standing for quadrupedal gait. The individual demographic characteristics and the MPTP treatment in the FOG monkey group (n=14) are presented in detail in Table 1, and those in the non-FOG monkey group (n=15) in Supplementary materials, Table S1.

NHP FOG occurred overtly in the “off” state with variable frequency in all 14 monkeys, but FOG was also observed in the “on” state in one monkey who had more severe parkinsonism. In this monkey, FOG was prominent in the “off” state, appeared again as the animal began to turn “on”, and continued during the peak of the levodopa response. *NHP FOG* in the “on” state seemed to be less frequent, shorter, and accompanied by more prominent trembling of the legs possibly due to stronger attempts to walk. “On”-state FOG in the monkey was seen with oral levodopa as well as subcutaneous injections of levodopa methyl ester. Subcutaneous doses were in the usual range used for tests in monkeys (levodopa methyl ester/ benserazide 50/12.5 to 150/37.5 mg) where the selected dose should produce a sub-maximal response or approximately 75% reduction of motor disability scores. Notably, in the monkey with “on”-state FOG, there was a significant residual disability at the peak of

the levodopa response that could not be reversed with higher doses. There were no dyskinesias during these “on”-states with *NHP FOG*. Regarding the low incidence of “on”-state FOG in this primate model, it should be noted that specific evaluations of responses to L-dopa for the appearance of FOG in the “on” state were not performed in the animals whose records were retrospectively reviewed. Individual behavioral data regarding the development of *NHP FOG* is presented in Table 2.

FOG associated tremor in MPTP-treated macaques

The tremulous movements in the legs that accompanied FOG episodes in the monkeys appeared similar to the regular oscillations that characterized FOG in PD patients (see videotapes in Supplementary materials). The recording of these episodes with accelerometry showed regular oscillations of short duration (Fig. 1), usually between 2 and 10 s, and variable frequencies from 4.6 to 9.7 Hz. Frequently, the tremor ended directly at the re-initiation of walking. However, in some instances 2–3 periods of tremor were seen before the end of freezing. Fig. 2A–D shows the typical leg tremor in 2 *NHP FOG* episodes from beginning to end. The mean rate of tremor from a total of 20 recordings was 7.07 Hz (± 1.47 STD), and the media of the rate distribution was 7.4 (Fig. 2E).

Phenotype correlates of NHP FOG

The characteristics of the animals analyzed in this study did not have major effects on the development of FOG with the exception of gender. The comparison between monkeys with FOG (n=14) and those without it (n=15), showed no statistically significant differences in macaque species, age (adults from 2 to 10 years old), or weight (2.8 and 9.9 kg). However, males were more likely to develop FOG than females (9 out of 12 males vs. 5 out of 17 females, $p=0.03$; Tables 1 and S1). The parameters of the MPTP treatment had no direct influence on the development of *NHP FOG*. There were no significant differences between FOG and non-FOG monkeys with regard to duration ($p=0.4$), number of injections ($p=0.2$), highest individual dose ($p=0.8$), or TCD ($p=0.2$) of MPTP (Tables 1 and S1).

The development of *NHP FOG* significantly correlated with the severity of parkinsonism and levodopa-induced dyskinesias. The comparison between FOG and non-FOG monkeys showed significant differences in MDS (median MDS: 19.8 versus 16.0, $p=0.01$) and TDS (median TDS: 10.3 versus 6.0, $p=0.04$, Table 3). Tables 2 and S2 show the individual data in each animal group. In FOG monkeys, MDS in the “off” state ranged from 12 to 29, and TDS in the “on” state ranged from 2 to 15. Maximal TDS was determined following a subcutaneous injection of L-dopa methyl ester plus benserazide (125/31.25 mg) or SKF82958 (1 mg/kg) to avoid response variability following oral dosing due to peripheral pharmacokinetics. Both drug treatments induced a full response with reductions of total MDS to 2 points or lower in most monkeys. In the FOG group, 12 monkeys were treated chronically with oral levodopa at total daily doses ranging from 75 to 325 mg/day, and the remaining 2 monkeys received subcutaneous SKF82958 at total daily doses of 1 mg/kg. The same treatments were used in monkeys who did not develop FOG. Statistical analyses of differences between the two groups are summarized in Table 3.

Discussion

Although others have alluded to freezing-like behavior in MPTP-treated monkeys (Jenner, 2003), to our knowledge this is the first detailed characterization of freezing of gait in this animal model. We found that the gait abnormality in monkeys meets the definition for FOG proposed for humans in several ways; it is episodic (where the animal is observed to ambulate fairly normally before and after an episode), of short duration (lasting seconds), and has the presence of rear leg trembling, reminiscent of human FOG. Recordings of these

movements demonstrated the presence of tremor during the freezing episode usually lasting less than 10 s and averaging a frequency of 7 Hz, which are comparable to the characteristics of the FOG tremor in patients (Moore et al., 2008), (Nutt et al., 2011). While these similarities support the FOG denomination, there are some species related differences and, thus, the phenomenon is described as *NHP FOG*. Profound akinesia was associated commonly with these freezing episodes in monkeys. Although this is described in patients with FOG (Nutt et al., 2011; Schaafsma et al., 2003), it appears to be more common in parkinsonian primates. As described in patients, *NHP FOG* was also found in the “on” state in one monkey; however, we could evaluate the “on” state specifically for the appearance of *NHP FOG* in only two monkeys of the prospective examination group that had freezing episodes in the “off” state. A larger sample of monkeys is needed to determine the actual frequency of *NHP FOG* in “on” state. Unlike PD patients, most animals had a tendency to sit when they experienced FOG. This difference is likely related to anatomical, postural and inherent gait differences (bipedal versus quadrupedal) between species. Aside from species related differences, there are also clear differences in the environment in which *NHP FOG* is seen, since most of the observations are made in caged animals. In that setting, the environmental factors that affect the occurrence of FOG in humans may not be present in primates. Regarding the animal’s intention to move it is important to note that the monkeys were incentivized by being offered food, as shown clearly at the beginning of video one. Nevertheless, the animal’s intention cannot be ascertained, and it is possible that the monkey is giving up the attempt to move temporarily and sits down. In both videos the monkey was moving before entering the freezing state and also has regained movement after the episode ended. Interestingly, in this study the frequency of freezing episodes in MPTP-treated monkeys was similar to that seen in advanced PD. In conclusion, the characteristics of this phenomenon and its consistent occurrence primarily in the “off” state in monkeys, are sufficiently similar to the FOG seen in patients to justify its use in further studies aimed at understanding the underlying pathophysiology.

In PD, FOG is most commonly encountered in patients with advanced motor disability, prolonged disease duration and levodopa exposure (Factor, 2008; Giladi et al., 2001a, 2001b; Nutt et al., 2011; Schaafsma et al., 2003). It is also correlated with the presence of dyskinesia (Giladi et al., 1992). The significant correlation of *NHP FOG* development with the more advanced parkinsonism in our monkeys, as shown by high disability scores and dyskinetic responses to levodopa, represents other clinical parallel of the primate model of FOG. Although FOG can occur early in PD, this is uncommon, 7% of patients with a diagnosis for 2 years or less (Giladi et al., 2001a, 2001b). Our data do not exclude that freezing may occur in monkeys with milder compromise, but indicate that modeling this disorder in MPTP-treated primates requires the consistency that was observed with more advanced parkinsonism.

There were no significant demographic differences between FOG and non-FOG groups to suggest that there was an inherent susceptibility to the development of this condition, with the exception of gender (64.3% male in the FOG group vs. 20.0% in the non-FOG group); a finding that would have to be confirmed with a larger number of monkeys and one not previously observed in patients (Factor et al., 2011). Regarding the administration of MPTP, neither TCD, nor the number of injections, nor the duration of treatment was significantly different between the FOG and the non-FOG group. This is not surprising since there is known variability in the susceptibility to MPTP in individual monkeys. Some may develop significant disability with shorter duration and lower doses, while others may require more aggressive treatment to reach comparable disability scores. The protocols for MPTP administration were individualized for each monkey, however, the common denominator for developing FOG was the presence of significant motor disability (median MDS=19.8 in the FOG group, and 16.0 in non-FOG group; $p=0.01$). Based on these findings and our

experience with the MPTP-treated monkey, we believe this model is reproducible by designing individualized protocols that yield this level of disability (MDS 20). The development of a primate model is an important tool for FOG research with potential uses for studies in various areas of pathophysiology and therapeutics. Electrophysiologic recordings in several brain regions of monkeys with FOG may contribute information to the underlying pathogenetic mechanisms of freezing. In addition, preclinical pharmacological trials or exploration of novel surgical targets for DBS using the primate model may significantly enhance the translational value of studies for FOG therapy.

Most studies agree that MPTP causes selective damage to the substantia nigra pars compacta (SNc) in primates. This lesion creates a state of dopamine deficiency that is associated with increased dopamine receptor sensitivity, and diminished striatal dopaminergic terminals and dopamine metabolites (3,4,-dihydroxyphenylacetic acid and homovanillic acid), but may leave other (non-dopaminergic) systems mostly intact (Jenner, 2003; Schmidt and Ferger, 2001). Although there are reports of locus ceruleus involvement in older monkeys (Forno et al., 1986), in practice, investigators turn to alternative toxins [6-hydroxydopamine (6-OHDA) or N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)] when there is an experimental need for true noradrenergic lesion in monkeys (Fornai et al., 1997, Mavridis et al., 1991). Since *NHP FOG* can develop following MPTP treatment, it is possible that dopamine deficiency plays a role. However, the advanced MPTP-induced lesion may also cause secondary changes in the basal ganglia network, the PPN, and other connected regions as the frontal premotor areas of the cortex. Thus, the MPTP-modeled *NHP FOG* may result from various mechanisms. The present data cannot address the neurotransmitter system abnormalities leading to FOG development in PD, but the finding of an available primate model is an important step forward.

Conclusions

Here, we showed that FOG can be replicated in advanced MPTP parkinsonian macaques with high frequency. We acknowledge that there may be phenomenological and, likely, pathophysiological differences between the “*NHP MPTP-FOG model*” and the human Parkinson’s disease-FOG. Nevertheless, this model can be of great utility when studying FOG in general, and the responses to a number of medical and surgical therapies for FOG in particular.

Videotaped observations of two monkeys are included in Supplementary materials. Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.expneurol.2012.07.021>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Variability in the tremor associated with FOG in the MPTP-treated monkey. The oscillatory movements recorded with an accelerometer placed on the back of the leg during 3 episodes of tremor associated with FOG as examples of variability are shown in the three traces. The raster on top of each trace shows the detection of full phase oscillations above the threshold. The frequency and duration of the tremor were calculated on the constructed raster. Tremor frequencies in these episodes were: 6.0, 7.8, and 6.6 Hz from top to bottom traces, respectively. Tremor durations in these episodes are shown next to each raster.

Fig 2.

Analysis of the evolution of FOG episodes in the MPTP-treated monkey. *A and B*, recordings of leg movements with an accelerometer placed on the back of the leg during the whole duration of FOG episodes (~15 s). Each episode (*A and B*) has regular oscillations corresponding to tremor before the end of freezing. The freezing episode showed in *B* also has some initial oscillatory movements that did not qualify as tremor according to the pre-established criteria. Also in *B*, the end of the freezing episode is followed by gait festination. The traces show raw accelerometry data. *C and D*, rate meters for the whole duration of the FOG episodes corresponding to *A* and *B*, respectively. The peaks correspond to the tremor periods towards the end of the freezing episode when walk restarts. Rate meters used the data produced after detection of full phase oscillations above the threshold. The graphs were constructed with a bin width of 500 ms, and smoothed using a Gaussian filter. *E*, distribution of FOG tremor frequencies. The graph shows the frequencies (Hz) found across 20 recordings of tremor, each in a separate FOG episode. The average rate in the recorded tremors was 7.07 Hz (± 1.47 STD).

Table 1

Demographics and MPTP Treatment in Monkeys with FOG.

Demographic data				MPTP administration				
Subjects	Species	Gender	Age ^a (years)	Weight ^a (kg)	TCD (mg/kg)	Single doses (mg/kg)	Number of injections	Duration (months)
1	MM	M	2	3.6	27	0.5–1	33	20
2	MF	M	N/A	4.6	1.75	0.75–1	2	1
3	MF	F	6	5	0.75	0.75	1	0
4	MF	F	4	4.1	1	0.5	3	1.5
5	MF	M	2	3.5	2.25	0.5–0.75	4	3
6	MM	M	2	3.1	12.5	0.75	15	12
7	MM	M	2	3.6	8.75	0.5–0.75	12	10
8	MM	M	2	3	11.5	0.5–1	13	18
9	MM	M	6.1	8.5	18.38	0.3–0.75	36	15
10	MM	M	6.2	9.9	5.65	0.5–0.65	11	5
11	MM	F	5	5.87	5.3	0.3–0.5	12	18
12	MM	F	2.6	5.2	3.65	0.1–0.75	13	5
13	MM	F	7.8	7.5	3.30	0.75	6	4
14	MF	M	N/A	5.6	2.25	1	3	1

Data correspond to monkeys who developed *NHP FOG*. There were interruptions in the administration of MPTP for periods longer than the usual weekly interval particularly in monkeys with longer duration of treatment.

^a Age and weight measurements were obtained at the time of the first administration of MPTP. TCD: total cumulative dose, MM: *Macaca Mulatta*, MF: *Macaca Fascicularis*, N/A: non-available.

Table 2

Behavioral Outcomes in Monkeys with FOG.

Subjects	MDS ^a	TDS ^b	Treatment	Dose ^c	Documented FOG	Data source
1	21	11	L-Dopa	300	Severe FOG and akinesia	Records, Video
2	20	10	L-Dopa	50	Severe FOG and severe akinesia	Records, video
3	26	10.5	L-Dopa	225	Severe FOG	Records, video
4	29	11	SKF82958	1	Frequent FOG with tremor	Records
5	21.4	12	SKF82958	1	Frequent FOG	Records
6	16.6	7	L-Dopa	150	Rapid development of FOG after 10th injection of MPTP (7.5 mg/kg TCD)	Records
7	19.5	10	L-Dopa	150	Rapid development of FOG with tremor after 8th injection of MPTP (6 mg/kg TCD)	Records
8	17	15	L-Dopa	325	Severe FOG, akinesia and FOG tremor	Records
9	12	2	L-Dopa	75	Occasional FOG	Direct examination
10	16	2	L-Dopa	75	Occasional FOG	Direct examination
11	21	8.5	L-Dopa	150	Frequent FOG	Records
12	19	9.5	L-Dopa	150	Frequent episodes of FOG, some with tremor	Records
13	25	15	L-Dopa	150	Frequent FOG	Records
14	19	13	L-Dopa	N/A	Occasional FOG	Records

Data correspond to monkeys who developed *NHP FOG*.

^aMDS is total motor disability score taken in the "off" state.

^bTDS is maximal total dyskinesia score taken at the peak "on" state after administration of levodopa methyl ester plus benserazide s.c. (125/31.25 mg).

^cDoses correspond to daily oral levodopa (Sinemet, mg) or daily s.c SKF82958 (mg/kg). N/A: non-available. Monkeys 9 and 10 were in the group of the prospective study (data source from direct examination). The remaining animals were all from the retrospective study. "Frequent FOG" denotes that the animal froze in the "off" state more than once during a single evaluation. "Occasional FOG" denotes FOG appearance during some but not all evaluations, and usually once during the evaluation.

Table 3

Comparisons between FOG and non-FOG monkeys.

	FOG (n=14)			Non-FOG (n=15)		
	Median	IQR ^a	^	Median	IQR ^a	p-value ^b
Age (years)	3.3	(2.0, 6.1)		4.6	(3.0, 5.7)	0.5853
Weight (Kg)	4.8	(3.6, 5.9)		5.1	(3.0, 6.9)	0.8102
MPTP treatment duration	5.0	(1.5, 15.0)		10.0	(4.0, 14.0)	0.4428
Number of MPTP injections	11.5	(3.0, 13.0)		15.0	(6.0, 24.0)	0.1816
MPTP highest single dose (mg/kg)	0.8	(0.8, 1.0)		0.8	(0.7, 1.0)	0.7833
MPTP TCD (mg/kg)	4.5	(2.3, 11.5)		10.0	(4.0, 12.0)	0.2385
MDS	19.8	(17.0, 21.0)		16.0	(15.0, 20.5)	0.0085 ^c
Dyskinesia score	10.3	(8.5, 12.0)		6.0	(0.0, 10.0)	0.0420 ^c
	n	%		n	%	p-value ^d
Species (<i>M. Mulatta</i>)	9	64.3		11	73.3	0.6999
Gender (males)	9	64.3		3	20	0.0253

The categorical variables species (*Macaca Mulatta* and *Macaca Fascicularis*) and gender present values for the group of *Macaca Mulatta* and males, respectively.

^aInterquartile range.
^bWilcoxon ranked sum test.
^cone-sided hypothesis testing.
^dFisher's exact tests.