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Natural history of motor symptoms in Parkinson's disease and the long-duration response to levodopa

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The natural pattern of progression of Parkinson's disease is largely unknown because patients are conventionally followed on treatment. As Parkinson's disease progresses, the true magnitude of the long-duration response to levodopa remains unknown, because it can only be estimated indirectly in treated patients. We aimed to describe the natural course of motor symptoms by assessing the natural OFF in consecutive Parkinson's disease patients never exposed to treatment (drug-naïve), and to investigate the effects of daily levodopa on the progression of motor disability in the OFF medication state over a 2-year period. In this prospective naturalistic study in sub-Saharan Africa, 30 Parkinson's disease patients (age at onset 58 ± 14 years, disease duration 7 ± 4 years) began levodopa monotherapy and were prospectively assessed using the Unified Parkinson's disease Rating Scale (UPDRS). Data were collected at baseline, at 1-year and 2-years follow-up. First-ever levodopa intake induced a significant improvement in motor symptoms (natural OFF versus ON state UPDRS-III 41.9 ± 15.9 versus 26.8 ± 15.1, respectively; $P < 0.001$). At 1-year follow-up, OFF state UPDRS-III score after overnight withdrawal of levodopa was considerably lower than natural OFF (26.5 ± 14.9; $P < 0$ .001). This effect was not modified by disease duration. At the 2-year follow-up, motor signs after overnight OFF (30.2 ± 14.2) were still 30% milder than natural OFF ($P = 0.001$). The ON state UPDRS-III at the first-ever levodopa challenge was similar to the overnight OFF score at 1-year follow-up and the two conditions were correlated ($r = 0.72$, $P < 0.001$). Compared to the natural progression of motor disability, levodopa treatment resulted in a 31% lower annual decline in UPDRS-III scores in the OFF state (3.33 versus 2.30 points/year) with a lower model's variance explained by disease duration (67% versus 36%). Using the equation regressed on pretreatment data, we predicted the natural OFF at 1-year and 2-year follow-up visits and estimated that the magnitude of the long-duration response to levodopa ranged between 60% and 65% of total motor benefit provided by levodopa, independently of disease duration ($P = 0.13$). Although levodopa therapy was associated with motor fluctuations, overnight OFF disability during levodopa was invariably less severe than the natural course of the disease, independently of disease duration. The same applies to the yearly decline in UPDRS-III scores in the OFF state. Further research is needed to clarify the mechanisms underlying the long-duration response to levodopa in Parkinson's disease. Understanding the natural course of Parkinson's disease and the long-duration response to levodopa may help to develop therapeutic strategies increasing its magnitude to improve patient quality of life and to better interpret the outcome of randomized clinical trials on disease-modifying therapies that still rely on the overnight OFF to define Parkinson's disease progression.

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Abbreviations: LDR = long-duration response to levodopa; SDR = short-duration response to levodopa; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

More than 50 years after its introduction by George C. Cotzias (Cotzias *et al.*, 1967), levodopa is still the most effective treatment for the motor symptoms of Parkinson's disease. Even at the dawn of the 2020s, several low-income countries worldwide are still living in a 'modern pre-levodopa era', as the access to dopaminergic treatment is still limited for the majority of patients with Parkinson's disease (Mokaya *et al.*, 2016; Okubadejo *et al.*, 2018; Lim *et al.*, 2019) and the initiation of levodopa often occurs several years after the onset of motor symptoms (Dotchin *et al.*, 2011; Cilia *et al.*, 2014).

To date, several studies have described the progression of Parkinson's disease motor symptoms and disability (Marras *et al.*, 2002; Reinoso *et al.*, 2015; Latourelle *et al.*, 2017). Nonetheless, available data are not conclusive, because the natural history of Parkinson's disease has been described in treated patients, with an obvious confounding symptomatic effect played by concomitant dopaminergic medications (Jankovic and Kapadia, 2001; Marras *et al.*, 2002, Evans *et al.*, 2011; Ding *et al.*, 2016; Simuni *et al.*, 2018). Population-based studies have shown a non-linear pattern of change in motor symptoms over time, which invariably includes a plateau corresponding to the response to levodopa and other dopaminergic drugs (Evans *et al.*, 2011; Reinoso *et al.*, 2015). The main limitation of the majority of these studies is the inability to measure the natural rate of progression of Parkinson's disease motor symptoms, as patients have been assessed in the ON medication state and attempts to measure this rate have been confounded by several pharmacokinetic and pharmacodynamic factors influencing individual responsiveness to dopaminergic treatment (Goetz *et al.*, 2000).

To overcome this issue, a number of randomized clinical trials investigating potential 'disease-modifying' therapies still use the change in the levodopa overnight OFF (or 'practical OFF' state) Unified Parkinson's Disease Rating Scale (UPDRS) score as clinical primary outcome measure to quantify and compare the differential progression of the degenerative process between the target therapy and the placebo. This measure is based on the conjecture that the overnight OFF UPDRS score can be considered a reliable marker reflecting the natural progression of dopamine cell loss. However, the daily intake of levodopa is associated with a sustained motor improvement that lasts several days after treatment discontinuation, which is a phenomenon called long-duration response to levodopa (LDR). Although this phenomenon was described by Cotzias *et al.* (1969) soon after the introduction of levodopa, the exact mechanisms have not yet been elucidated (Nutt and Holford, 1996; Albin and Leventhal, 2017; Nagao *et al.*, 2019). While the short-duration response (SDR) is closely related to levodopa plasma pharmacokinetics, the LDR seems to be associated with more complex pharmacodynamic mechanisms that take days to build up after levodopa initiation (Anderson and Nutt, 2011). It has been suggested that the magnitude of motor response to levodopa results from the combination of the LDR plus the SDR plus endogenous dopamine production by residual dopamine neurons (Nutt and Holford, 1996; Anderson and Nutt, 2011; Nagao *et al.*, 2019). Understanding the mechanisms underlying the LDR and their relationship with Parkinson's disease progression is essential to design clinical trials on potential 'disease-modifying' therapies (Lang and Espay, 2018).

In this scenario, there are two major questions that are still unanswered: (i) what is the 'true' rate of progression of Parkinson's disease? and (ii) is the overnight OFF state a reliable marker of disease progression in patients on stable levodopa? We took advantage of the differences in healthcare and treatment availability in the unique sub-Saharan African setting to observe the natural progression of the disease by collecting clinical data of untreated patients reaching the most advanced and disabling stages of the disease and by assessing the response to levodopa monotherapy in the long-term (Cilia *et al.*, 2014). The objectives of the present study were (i) to investigate the natural progression of Parkinson's disease motor symptoms in a cohort of consecutive drug-naïve patients assessed at different disease stages using a cross-sectional design; and (ii) to assess the response to levodopa monotherapy and the potential changes in disease progression while on stable treatment using a 2-year prospective

naturalistic study design fulfilling present-day standards of assessment. Specifically, we focused on the comparison between natural motor disability (natural OFF) versus overnight OFF state on levodopa. The response to levodopa of axial motor features that are generally considered to be levodopa-resistant, such as dysphagia, postural instability and falls, was an additional aim of the study.

Materials and methods

Participants

In the present prospective naturalistic study, we included all drug-naïve patients consecutively attending three out-patient clinics in different regions of Ghana and one patient visited in a rural region of Zambia, diagnosed with idiopathic Parkinson's disease (Postuma *et al.*, 2015) by a single neurologist experienced in movement disorders (R.C.). Onset of the disease was defined as the first appearance of any motor symptom, as reported by the patient, a family member or a clinician. In case of any doubt or uncertainty, we adopted a recall technique tailored to major events in the patient's life (Cilia *et al.*, 2014).

As we aimed to describe the natural progression of Parkinson's disease motor symptoms and their response to levodopa monotherapy in the long-term, we included only patients never exposed to any antiparkinsonian therapy (drug-naïve) at the baseline assessment and who had at least one follow-up visit after 12 ± 3 and 24 ± 6 months from baseline. If a longer follow-up was available, clinical data at the last visit were also included. Patients with disabling symptoms were admitted to hospital to ensure supervision. In these cases, clinical data were additionally recorded 24 h after the initiation of levodopa therapy. To ensure homogeneous assessment of motor symptoms and avoid any ascertainment bias, we limited the analysis to patients assessed by the same neurologist (R.C.) over time.

Clinical work-up included the UPDRS parts II, III and IV (Fahn and Elton, 1987) and Hoehn and Yahr staging (Hoehn and Yahr, 1967). Major milestones of progression of motor symptoms were assessed using the part II (dysphagia, item 7 score $\geqslant 2$; falls, item 13 score $\geqslant 52$) and part III (postural instability, item 30 score $\geqslant 2$) UPDRS items (Cilia *et al.*, 2014). We calculated a distinct score for non-dopaminergic motor symptoms, according to the criteria proposed by Levy *et al.* (2000). Acute response to levodopa was assessed at baseline and follow-up visits using dispersible levodopa/benserazide (100/25 mg), providing either a 150-mg or 200-mg levodopa dose, according to body weight ($\leqslant 70$ kg or >70 kg, respectively). After the first-ever levodopa intake in the morning, patients were administered two additional levodopa doses on that day (Day 1) and then started levodopa at 100 mg three to four times daily from the subsequent day (Day 2), and then continued adhering to this dosage regimen. If further treatment was required, additional 50 or 100 mg of levodopa could be added during the follow-up, as appropriate, to achieve adequate control of motor symptoms. In case of hospitalization, patients took additional doses of 100 or 150 mg three to four times (according to body weight and to motor response) on Days 1 and 2. At each follow-up visit, all patients were assessed after at least 12-h overnight withdrawal of levodopa (overnight OFF) and 90 min after levodopa intake (ON state). At each visit at all time points, the presence of motor fluctuations (including the approximate duration of the ON state following a single levodopa dose) and dyskinesia was assessed by recall and prolonged direct observation, and time of their first occurrence was recorded. All patients were observed by the neurologist for a minimum of 4 h after the administration of dispersible levodopa/benserazide to monitor motor response (prolonged direct observation aimed to detect subtle wearing OFF and dyskinesias and to minimize any recall bias). To minimize recall bias and linguistic barriers we carefully collected information with the help of younger English-speaking first-degree family members and caregivers, as well as co-authors of the study and nurses of each clinic. In case of hospitalization, patients were examined at least four times per day: right before taking levodopa (to monitor wearing OFF and end-of-dose dyskinesia) and 90 min after (to monitor peak-dose dyskinesia) on Day 0 (baseline) and Day 1 (24 h). Motor fluctuations were defined as predictable wearing OFF, unpredictable ON–OFF fluctuations and sudden OFF periods according to UPDRS part IV (Fahn and Elton, 1987). Concerning motor fluctuations, we interviewed patients using the following definition: 'A generally predictable recurrence of motor or non-motor symptoms that precedes a scheduled dose and usually improves with the next dose of antiparkinsonian medication', as agreed by the 'Wearing OFF working group' in September 2004 (Chou *et al.*, 2018). Dyskinesias were defined as abnormal involuntary movements, including chorea and dystonia, that could occur at peak dose or be diphasic; OFF-related dystonia was not included.

Statistical analysis

Descriptive statistics of continuous variables and categorical variables were reported as mean and standard deviation (SD) and as counts and percentages, respectively. Paired data comparisons between the different time points were performed using the Student's *t*- (continuous variables) or the McNemar's (categorical variables) tests. The correlation between UPDRS-Part III OFF state and disease duration at the different follow-up assessments (baseline, 1 year and 2 years) was investigated through Pearson's statistics and the contribution of disease duration to deterioration of motor symptoms was quantified using general linear regression model ($y = ax + b$) and calculating the variance explained by the model (R^2). The slope (a) of the related models was then compared using the *t*-test. Change in UPDRS-Part III OFF state over time was also investigated using general linear model for repeated measurements testing for the interaction with disease duration (<7 versus $\geqslant 7$ years).

Using the equation regressed on baseline data—and explaining the association between UPDRS-III natural OFF and disease duration—we also predicted the natural OFF at 12-month and 24-month follow-up visits in different disease duration strata (tertiles of distribution: $\leqslant 5$, 6–10 and >10 years) of our study population in order to estimate the absolute magnitude of the LDR (difference between the natural OFF state and the overnight OFF state) and its contribution to the total motor benefit of levodopa (percentage of change in the difference between the natural OFF state and the ON state). The SDR is the shift between the overnight OFF and the ON state.

Finally, linear regression was also used to investigate variables predicting the response to acute levodopa challenge at baseline and at 1-year follow-up as well as the change between natural OFF versus the overnight OFF at 1-year follow-up. Independent

non-collinear variables showing an association at univariate analysis ($P < 0.10$) were then included in multivariate models, as appropriate.

All statistical analyses were performed using STATA statistical software release 15.1 (Stata Corporation, College Station, TX) setting the level of significance at a two-tailed P-value < 0.05.

Data availability

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request and after its formal approval by the Ethics Committee.

Ethics

This study was performed in agreement with the principles of the Declaration of Helsinki and approved by the local Ethics Committee. We obtained written informed consent, which was translated into local dialect whenever required and/or was provided by a first-degree relative in cases of need.

Results

According to our *a priori* criteria, 30 patients with idiopathic Parkinson's disease (63.3% males, mean age 64 years) were included in the study. The flow chart is shown in Supplementary Fig. 1. All had complete 1- and 2-year follow-up data and eight had an additional 4-year follow-up assessment. Demographic and clinical features of the primary study population are summarized in Table 1, while those of the subgroup of patients assessed at 4 years are reported in Supplementary Table 1.

Motor features at baseline

According to cross-sectional data analysis, untreated Parkinson's disease patients showed an annual decline in UPDRS-III scores of 3.33 points per year of disease duration, with 67% of the variance being explained (R^2) by the model (Fig. 1). After a mean (SD) of 7 (4) years from the onset of motor symptoms, ~40% of patients had developed postural instability and falls, while 17% had clinically relevant dysphagia and freezing of gait (UPDRS score $\geqslant 2$ for items 7 and 14, respectively). Baseline features and milestones are detailed in Table 1.

Response to levodopa

Short-duration response to levodopa

The first-ever intake of levodopa performed at a mean (SD) levodopa dose of 158.9 (33.5) mg (2.8 ± 0.5 mg/kg/day) produced an UPDRS-III score improvement of ~40%. First-ever levodopa challenge was generally well tolerated (mild nausea in four patients; none experienced vomiting, hypotension or other clinically remarkable side effects) and the subsequent assessment in the ON state after 90 min was always feasible. Motor response to single-dose intake of levodopa showed a mild but steady and significant increase at the 1- and 2-year follow-ups (Table 1). With multivariate linear regression analysis (including the following variables, according to univariate association: the natural OFF state UPDRS-III score, levodopa dose, age at onset, disease duration), the response to the first-ever levodopa challenge (percentage of change) at baseline was inversely associated with Parkinson's disease duration [for 1-year increase, β coefficient (standard error), –2.3% (0.8); $P = 0.007$]. At 1-year follow-up, the response to levodopa (individual change between overnight OFF and ON) was correlated only to the ON state UPDRS-III score ($P < 0.001$), whereas there was no association with disease duration, daily levodopa dose adjusted by body weight, natural OFF score and the ON state UPDRS-III score at the first-ever levodopa challenge.

Interestingly, the response to the first-ever levodopa challenge was significantly associated with motor symptoms in the ON state (the higher the former the lower the latter) at the 1-year follow-up ($P = 0.022$). There was no difference between mean UPDRS-III scores in the ON state after the first-ever levodopa challenge and the mean UPDRS-III score at overnight OFF at 1-year follow-up ($P = 0.89$; Fig. 2A), as further evidence supporting the strong correlation between the two scores [$r = 0.72$ (95%CI 0.48–0.85), $P < 0.001$; Fig. 3].

In a subgroup of nine patients observed 24 h after the initiation of levodopa treatment (admitted to hospital at the baseline visit due to disabling symptoms), we observed a significant improvement in motor performance at overnight OFF compared to the natural OFF (mean change 27.3%, $P < 0.001$) as well as in the ON state (Day 2 versus Day 1, $P = 0.022$) UPDRS-III scores after 24 h of levodopa therapy, including the 'non-dopaminergic' score ($P = 0.006$) and the Hoehn and Yahr stage ($P = 0.047$) (Supplementary Fig. 2A and Supplementary Table 2). At 24 h the LDR accounted for 62% and the SDR for 38% of the total motor response provided by levodopa. Interestingly, the overnight OFF UPDRS-III score on Day 2 was similar to the ON state score after the first-ever levodopa intake on Day 1 ($P = 0.83$; Supplementary Fig. 2A) with a significant correlation between the two [$r = 0.94$ (95% confidence interval, CI 0.74–0.99), $P = 0.002$].

Long-duration response to levodopa

After 1 year of treatment with levodopa, two-thirds of patients experienced diurnal motor fluctuations and about one-third had dyskinesias (Table 1). All patients improved after the initiation of levodopa and all those who developed motor fluctuations had predictable OFF states, none reporting unpredictable OFF. At overnight OFF, motor performance was much better than the natural OFF at both the 1-year and 2-year follow-ups, with a mean UPDRS-III change of 38% and 29%, respectively (Table 1 and Fig. 2A). LDR persisted even after 4 years ($n = 8$), the mean overnight OFF score being 24% lower than at baseline ($P = 0.008$, Supplementary Fig. 2B and Supplementary Table 1). General linear model for repeated measurements showed that this effect was not modified by disease duration (no interaction

Table 1 Features of patients with Parkinson's disease with complete baseline (T0), 1-year (T1) and 2-year (T2) follow-up data

Features	Baseline	1-year Follow-up	2-year Follow-up	*P*-value* T0 versus T1	*P*-value* T0 versus T2	*P*-value* T1 versus T2
Patients, *n* (males/females)	30 (19/11)	30 (19/11)	30 (19/11)	–	–	–
Age at PD onset, years, mean (SD) [range]	57.8 (13.2) [22–81]			–	–	–
Age at visit, years, mean (SD) [range]	64.0 (13.3) [42–93]	65.0 (13.3)	66.0 (13.3)	–	–	–
Body weight, kg, mean (SD)	57.2 (10.7)	57.2 (9.2)	59.0 (10.4)	0.084	0.24	0.53
Education, years, mean (SD) [range]	7.6 (5.9) [0–15]			–	–	–
Disease duration, years, mean (SD) [range]	7.1 (3.9) [2–20]	8.1 (3.9) [3–21]	9.1 (3.9) [4–22]	–	–	–
Follow-up duration, months, mean (SD)	–	11.6 (3.3)	23.8 (5.2)	–	–	–
UPDRS II – OFF, mean (SD)	14.7 (9.5)	11.0 (8.4)	12.6 (8.6)	**<0.001**	**<0.001**	**0.034**
Dysphagia, mean (SD), score	0.5 (1.0)	0.2 (0.5)	0.2 (0.5)	0.15	0.18	0.33
n (%) with dysphagia[a]	5 (16.7)	1 (3.3)	1 (3.3)	0.13	0.13	1.00
Falls, mean (SD), score	1.0 (1.2)	0.5 (1.0)	0.6 (0.9)	**0.024**	**0.025**	0.66
n (%) with falls[a]	12 (40.0)	6 (20.0)	6 (20.0)	**0.041**	**0.041**	1.00
Freezing of gait, mean (SD), score	0.5 (1.0)	0.2 (0.5)	0.4 (0.7)	**0.005**	**0.016**	0.66
n (%) with freezing of gait[a]	5 (16.7)	1 (3.3)	4 (13.3)	0.13	1.00	0.25
UPDRS III – OFF, mean (SD) [range][b]	41.9 (15.9) [13–80]	26.5 (14.9) [10–62]	30.2 (14.2) [12–62]	**<0.001**	**<0.001**	**0.010**
Change from baseline, mean (SD), %	–	37.9 (20.7)	28.8 (16.3)	–	–	**0.002**
Speech, mean (SD), score	1.7 (0.9)	1.2 (0.9)	1.3 (0.7)	**0.015**	**0.041**	0.57
Arise from the chair, mean (SD), score	1.5 (1.3)	0.8 (1.1)	0.9 (1.1)	**<0.001**	**0.005**	0.10
Posture, mean (SD), score	1.5 (1.0)	1.1 (0.8)	1.3 (0.7)	**0.012**	0.44	**0.030**
Gait, mean (SD), score	1.6 (1.1)	1.1 (1.1)	1.3 (1.1)	**0.010**	0.23	**0.017**
Postural stability, mean (SD), score	1.2 (1.2)	0.8 (1.2)	1.0 (1.1)	**0.025**	0.15	0.16
n (%) with postural instability[a]	13 (43.3)	5 (16.7)	7 (23.3)	**0.013**	**0.041**	0.48
Non-dopaminergic score, mean (SD)[c]	7.4 (5.0)	5.0 (4.4)	5.9 (4.0)	**<0.001**	**0.035**	**0.003**
UPDRS III – ON, mean (SD)	26.8 (15.1)[d]	15.9 (10.5)	17.3 (10.3)	**<0.001**	**<0.001**	0.62
Response to levodopa, mean % (SD)	38.8 (15.1)[d]	41.3 (19.0)	44.2 (17.4)	0.85	**0.029**	**0.005**
Levodopa daily dose, mg/day	–	358 (102)	405 (122)	–	–	**0.014**
Levodopa dose weight-adjusted, mg/kg/day	–	6.2 (1.7)	7.0 (2.0)	–	–	**0.022**
Duration of single dose ON, h, mean (SD)[e]	–	4.0 (1.1)	3.8 (0.9)	–	–	0.26
n (%) with motor fluctuations[f]	–	20 (66.7)	26 (86.7)	–	–	**0.041**
n (%) with dyskinesias	–	11 (36.7)	13 (43.3)	–	–	0.48
Hoehn and Yahr stage, mean (SD)	2.7 (0.9)	2.3 (0.6)	2.5 (0.6)	**0.006**	0.11	**0.014**

IQR = interquartile range (25th–75th percentile); PD = Parkinson's disease; SD = standard deviation.

[a]Dysphagia (item 7), falls (item 13), freezing of gait (item 14) and postural instability (item 29) were considered clinically meaningful when the score was $\geqslant 2$.

[b]OFF is considered the drug-naïve score at baseline and the overnight medication OFF (> 12 h from the last levodopa intake) at follow-up visits.

[c]Sum of UPDRS items 18-27-28-29-30 as defined by Levy *et al.* (2000).

[d]UPDRS motor score recorded 90 min after the first-ever administration of levodopa/benserazide at ~3 mg/kg of body weight (2.8 ± 0.6 mg): mean (SD) levodopa dose 158.9 (33.5). First-ever levodopa challenge was generally well tolerated [four patients (13.3%) complained about nausea, but none had vomiting or hypotension or other side effects] and the assessment in the ON state had never been compromised.

[e]Mean duration of ON time after the intake of the morning dose of levodopa as recorded at the follow-up visit. This period is approximated as it has been calculated using both the patient-caregiver's report and the direct observation for at least 4 h after levodopa intake by the neurologist.

[f]Concerning patients who had not developed motor fluctuations, we recorded only one UPDRS part II score; likewise, UPDRS part III scores were similar at overnight OFF and at ON state.

*By Student's *t*-test for paired data or McNemar's test.

elicited). Levodopa therapy significantly improved the activities of daily living (ADL, UPDRS-II score), freezing of gait, axial motor features (i.e. speech, rising from the chair, posture, gait, postural stability and falls). These improvements are globally summarized by the change in the 'non-dopaminergic' score and the Hoehn and Yahr stage (Table 1 and Fig. 2B). Individual discrepancies in the UPDRS-III score between the natural OFF versus the overnight OFF at follow-up were not explained by any potentially relevant demographic or clinical variable (daily levodopa dose adjusted by body weight, disease duration, age and age at onset, response to levodopa). Compared to the natural progression of motor symptoms, levodopa treatment resulted in a 31% lower annual decline in UPDRS-III OFF scores [3.33 versus 2.30 points/year; (for slope comparison, *P*-value = 0.17)] (Fig. 1A) with a lower variance explained by disease duration (R^2, 67% versus 36%). On the other hand, the annual decline in UPDRS-III OFF scores after 2 years was substantially similar to the one observed at the 1-year follow-up visit [2.63 points/

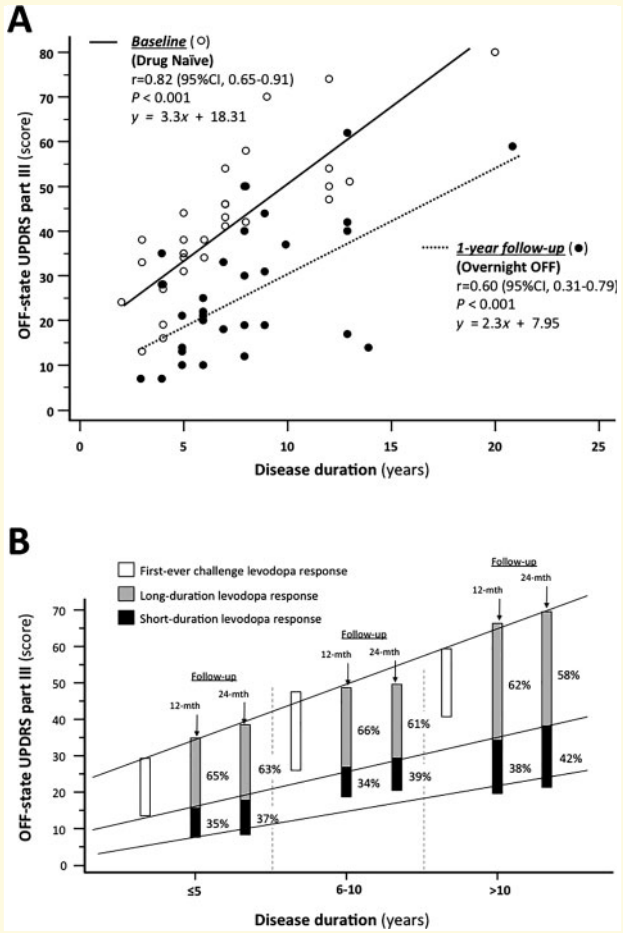

Figure 1 Progression of Parkinson's disease motor disability according to levodopa status (drug-naive versus stable therapy) and response (LDR and SDR). (**A**) Scatterplot of UPDRS motor score at natural OFF (solid line) and at 1-year follow-up after overnight withdrawal of levodopa (dotted line). Data are described according to Pearson's statistic [correlation coefficient (*r*) and *P*-value] and linear regression analysis (trend line and equation). (**B**) Estimation of 12-month and 24-month LDR (grey bars) and SDR (black bars) to levodopa in different disease duration strata (tertiles of distribution). LDR is calculated as the difference between the natural OFF state (predicted from disease duration using the equation regressed from baseline cross-sectional data analysis) and the overnight OFF state, while the SDR is the shift between the overnight OFF and the ON state. White bars represent the changes in UPDRS-III between the natural OFF and the ON state at the first ever levodopa challenge. Trend lines of natural OFF state, overnight OFF and ON state are also included.

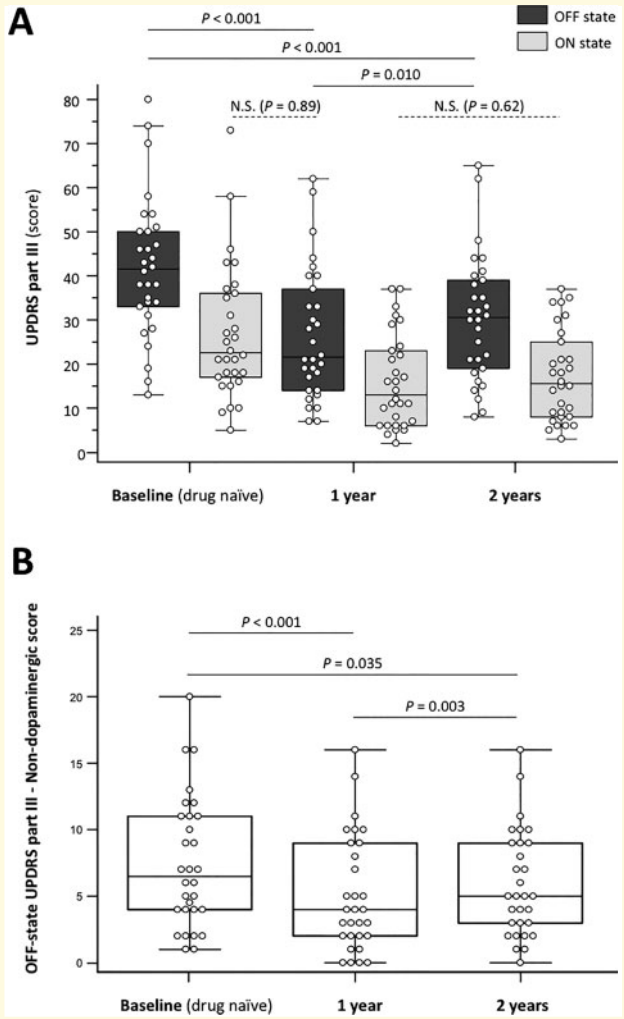

Figure 2 Response to levodopa at the first-ever intake (baseline) and at the follow-up visits. Box and whisker plots of UPDRS motor score (**A**) in the OFF state (dark boxes) and ON state (light boxes) and non-dopaminergic score of the UPDRS-III in the OFF state (**B**) at the baseline visit and at 1-year and 2-year follow-ups. The box represents the median value (middle line) and the interquartile range (IQR; 25–75th percentile). N.S = not significant.

year, $r = 0.72$ (95%CI, 0.49–0.86); $P < 0.001$]. Using the equation regressed on baseline data ($y = 3.3x + 18.31$) (Fig. 1B), we predicted the natural OFF at 12-month and 24-month follow-up visits and estimated that the magnitude of the LDR ranged approximately between 60% and 65% of total motor benefit (difference between the natural OFF state and the ON state) provided by levodopa, independently of disease duration ($P = 0.13$). Therefore, the relative contribution of the LDR to total motor benefit provided by levodopa remains greater than the SDR, even when Parkinson's disease duration is longer than 10 years. As the patient with the longest disease duration and the youngest age at onset might appear an outlier, we reran all correlation and regression models after exclusion of this case, but all the associations were consistent with the first set of analyses.

As our first paradigmatic case (Supplementary Video 1, segment 1), we describe a 42-year-old patient with a 20-year history of disease, never treated. He presented with diffuse resting tremor and very severe muscle rigidity with painful dystonic postures; he was unable to stand unassisted. He had dysphagia likely due to both neck hyperextension and disease progression itself (UPDRS-III 80/108, Hoehn and Yahr 5/5). The initiation

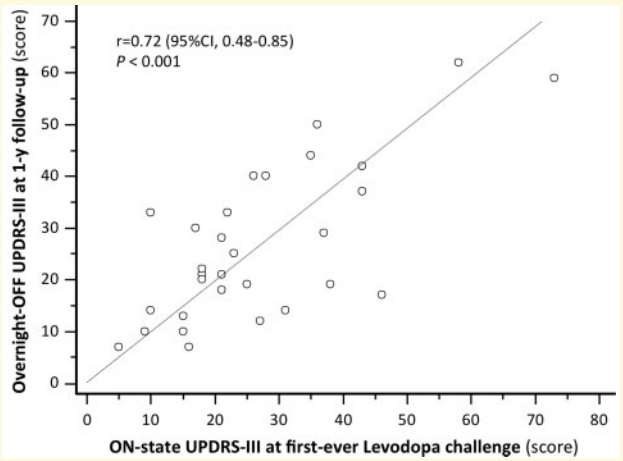

Figure 3 Relationship between the SDR at baseline and the LDR at follow-up. Correlation between the SDR at the first ever levodopa challenge (ON state UPDRS-III score) and the LDR at 1-year follow-up (overnight OFF).

of levodopa (400 mg/day) promptly improved motor symptoms and after a few days he could walk unassisted. At 9-month follow-up, he had 3-h wearing-off and mild levodopa-induced dyskinesias; tremor was absent, gait and balance were almost normal in the levodopa ON state (UPDRS-III 28/108; Hoehn and Yahr 2/5). After overnight withdrawal of levodopa, he was still able to stand up and walk unassisted, though limb tremor was moderate, his gait was shuffling with inconstant freezing, and he was not able to recover at pull-test (UPDRS-III 52/108; Hoehn and Yahr 3/5).

The duration of the LDR: effects of temporary withdrawal of levodopa

This follow-up observational study stems from a twin project started in Ghana with the aim of providing levodopa medication free of charge to all patients diagnosed with Parkinson's disease (Cilia *et al.*, 2011, 2014), which is still ongoing successfully. Shipping of levodopa supplies occurs approximately every 4–6 months according to local needs (number of patients on levodopa therapy and individual daily dosage regimen) and independently of our scheduled visit. On a few occasions we experienced delays between the shipping of levodopa supplies from Italy and the actual distribution to patients, due to different reasons (clearance delay at customs, patients not attending the scheduled visit, etc.). Therefore, during the follow-up, eight patients experienced a temporary withdrawal of levodopa for a period ranging from 7 to 30 days. Keeping in mind that all patients were on levodopa monotherapy, we found a sustained LDR 7 days after of withdrawal in patients with a 15-year history of Parkinson's disease, with UPDRS-III scores still 32–55% better than the baseline (including axial features, such as postural stability) despite the 3–5 years elapsed from the baseline assessment. On the other hand, temporary withdrawal of levodopa for time intervals longer than 15 days, 8 years after the onset or for just 7 days after 20 years of Parkinson's disease, was associated with return of motor performance similar to baseline. Individual patient data are reported in Table 2.

In our second paradigmatic case (Supplementary Video 1, segment 2), we describe a 69-year-old patient with a 12-year history of untreated Parkinson's disease and severe motor disability, who showed a dramatic improvement 24 h after the initiation of levodopa (UPDRS-III from 50/108 at baseline, Hoehn and Yahr 4/5 to 18/108, Hoehn and Yahr 2/5 in the ON state). Although he developed early motor fluctuations and dyskinesias, his motor performance in the overnight OFF state at the 2-year follow-up was still much better than at baseline (UPDRS-III 14/108, Hoehn and Yahr 2/5). After 3 years, although he experienced a 7-day withdrawal of levodopa, his motor performance was still better than the baseline, as he could rise from a chair without help and recover at pull-test (UPDRS-III 34/108, Hoehn and Yahr 2.5/5; Table 2).

Progression of motor symptoms on levodopa

In addition to previous considerations on the yearly decline in UPDRS-III OFF scores (Fig. 1), we also report that, compared to 1-year follow-up, OFF state UPDRS-II and -III scores also worsened at 2 years ($P = 0.010$), despite the increase in daily levodopa dosage, while the UPDRS-III in the ON state remained unchanged ($P = 0.62$). The prevalence of subjects who developed motor fluctuations significantly increased. Nonetheless, OFF state motor performance (including 'non-dopaminergic' axial features and the freezing of gait) and the ADL after 2 years were still significantly better than at baseline (Table 1 and Fig. 2). At 4-year follow-up, UPDRS-III scores in the overnight OFF and in the ON state remained significantly lower than at baseline ($P = 0.008$ and $P = 0.048$, respectively), despite the UPDRS-III worsening between the 1- and 4-year follow-up visits ($P = 0.013$) (Supplementary Fig. 2B). At this time point, the UPDRS-II score, the 'non-dopaminergic' score and the Hoehn and Yahr stage were similar to baseline (Supplementary Table 1).

Discussion

Our 10-year clinical and research experience on Parkinson's disease in sub-Saharan African countries has provided us with the unique opportunity to obtain insights on the natural progression of the disease without the confounding effect of dopaminergic medications and to observe the effects of levodopa therapy at different stages of the disease (Cilia *et al.*, 2011, 2014). In our previous report, we provided evidence showing that delaying levodopa does not delay the onset of motor fluctuations and dyskinesias: it was not the duration of levodopa therapy that was associated with

Table 2 Relationship between temporary levodopa withdrawal and individual demographic and clinical features

Patient	Gender	Age, years	PD duration at baseline, years	PD duration at withdrawal, years[a]	Duration of withdrawal (days)	UPDRS-III score				Levodopa daily dose at withdrawal	
						Baseline OFF	Withdrawal OFF	Change (%)[b]	Time from baseline to withdrawal, years	mg/day	mg/kg/day
1	Male	74	6	10	7	31	12	−38.7	4	400	8.0
2	Male	76	5	11	7	31	14	−54.8	6	400	7.5
3[c]	Male	73	12	15	7	50	34	−32.0	3	600	9.7
4	Male	42	20	21	7	80	72	−10.0	1	400	6.7
5	Female	78	8	10	15	50	47	−6.0	2	300	6.5
6	Male	53	4	8	21	28	35	+25.0	4	400	4.6
7	Male	69	5	9	30	34	38	+11.8	4	400	6.7
8	Male	66	5	8	60	35	39	+11.4	3	400	6.5

[a]Corresponding also to the duration of follow-up and the duration of levodopa therapy.
[b]UPDRS-III score change from baseline OFF to withdrawal OFF. Negative % indicates better performance than baseline OFF.
[c]This patient is shown in Supplementary Video 1, segment 2.
PD = Parkinson's disease.

the so-called 'levodopa-induced' motor complications, but it was the progression of the disease as well as the daily levodopa dose (Cilia *et al.*, 2014; Fox and Lang, 2014). It has been suggested that the emergence of motor complications in patients on levodopa therapy may be related to the progressive decline in the LDR with relative increase in the magnitude of the SDR with advancing disease. However, the relationship between the progression of Parkinson's disease motor disability and the LDR in the pathophysiology of motor fluctuations and dyskinesia has not been fully elucidated (Wider *et al.*, 2006; Anderson and Nutt, 2011; Albin and Leventhal, 2017). We herewith focus our attention on both the short- and long-duration response to levodopa in untreated individuals at different disease stages.

The 'natural history' of motor disability in untreated Parkinson's disease

In the present study, we focused on the progression of motor symptoms in a cohort of Parkinson's disease patients with a variable duration of motor symptoms, which were assessed before the initiation of levodopa monotherapy and thereafter regularly for at least 2 years. Compared to the natural progression of motor disability, levodopa treatment resulted in a 31% lower annual decline in UPDRS-III scores in the OFF state (3.33 versus 2.30 points/year) with a lower variance explained by disease duration (67% versus 36%). Although the slopes of the two linear regression models were not significant, we have estimated that doubling the sample size would have probably resulted in a significant difference, as is also suggested by the difference in the variance explained by the models. These data suggest that the relative contribution of the LDR to total motor benefit provided by levodopa is greater than what was estimated in previous models (Albin and Leventhal, 2017; Nagao *et al.*, 2019), especially at advanced Parkinson's disease stages. In agreement with recent evidence (Espay, 2019; Verschuur *et al.*, 2019), the present data do not provide evidence that levodopa has an impact on the underlying progression of the neurodegenerative process, but merely suggest that its symptomatic effect delays the natural progression of motor disability, acting through the complex mechanisms of the LDR. This interpretation is supported by the reappearance of a motor disability similar to baseline after a washout period longer than 15 days, which is in line with the notion that the LDR may last several weeks (Olanow, 2015; Leal Rato *et al.*, 2020). Although limited by the cross-sectional design, our estimate of the 2.3 points/year annual worsening of the UPDRS-III scores of levodopa-treated Parkinson's disease patients in sub-Saharan African countries is similar to the rate described in European patients, which ranges from 2.24 (Evans *et al.*, 2011) to 2.46 (Velseboer *et al.*, 2013). This strengthens the validity of the present findings and confirms the similarities in terms of Parkinson's disease features and levodopa response between the sub-Saharan African and the European populations (Cilia *et al.*, 2014). In a recent study reporting the progression of clinical and imaging markers in a cohort of early Parkinson's disease over a 5-year period, the annualized change in putaminal dopamine transporter density was higher at 1-year follow-up than at the 2- and 4-year assessments (Simuni *et al.*, 2018). These findings are in line with neuropathology data showing a rapid decline in nigrostriatal terminals 4 years after diagnosis (Kordower *et al.*, 2013) and support the notion of a 'floor effect' for change in striatal dopamine transporter density making this *in vivo* imaging biomarker not adequate for the investigation of disease progression beyond mild-to-moderate stages (Strafella *et al.*, 2017). Therefore, neither the overnight OFF state nor *in vivo* dopamine transporter imaging reliably reflect the progression of Parkinson's disease when the appearance of motor fluctuations takes over the 'honeymoon' phase of levodopa.

How the long-term response to levodopa affects the 'natural' progression of motor symptoms

We provide evidence supporting persistent LDR independently of disease duration, even in the most advanced stages of Parkinson's disease. Overall, although daily levodopa monotherapy was associated with the development of motor fluctuations and dyskinesias at follow-up in most patients, motor performance in the overnight OFF was invariably less severe than at baseline, even when patients were followed for a time interval up to 4 years. It is to be emphasized that in this cohort of Parkinson's disease patients, the symptomatic effect of levodopa was not influenced by any other dopaminergic enhancer (e.g. dopamine agonist, MAO-B or COMT inhibitors). The magnitude of this effect is well shown by the Parkinson's disease patient whose first-ever intake of levodopa occurred 20 years after onset. The LDR has been further confirmed by the better motor performance we observed in a few patients with Parkinson's disease duration ranging between 10 and 15 years after 7 days of accidental temporary discontinuation of levodopa treatment. Our experience in patients with advanced Parkinson's disease expands the experience gained with patients in the early stages of the disease from the ELLDOPA study (Fahn *et al.*, 2004), supporting the notion that the LDR has a major impact on the interpretation of the outcome of disease-modifying clinical trials. To date, the duration of withdrawal that is needed to estimate the magnitude of LDR in relation to the rate of disease progression and the magnitude of dopamine cell loss is still unclear (Anderson and Nutt, 2011; Olanow, 2015; Nagao *et al.*, 2019), especially considering the common use of add-on medication to levodopa. In a previous study in early Parkinson's disease patients, it was calculated that the mean half-life of the decline in motor benefit after treatment discontinuation amounts to ~8 days for levodopa plus the dopamine-agonist bromocriptine (Hauser and Holford, 2002). The present data on patients on levodopa monotherapy are not biased by any confounding adjunctive dopaminergic therapy (Nutt *et al.*, 2002; Albin and Leventhal, 2017). Although the cohort of patients who experienced a prolonged discontinuation of levodopa was relatively small and the washout period was variable, the present data and our personal experience on Parkinson's disease in sub-Saharan Africa suggest that a significant clinical benefit due to the LDR may last several days even in patients with advanced Parkinson's disease. Therefore, we suggest that estimating the LDR by using the overnight OFF motor score is likely not to represent the real magnitude of this effect. It is important to emphasize that the LDR persists throughout the course of Parkinson's disease even during the 'worst OFF' condition experienced by patients in their everyday life and that it accounts for about one-third of the variance, while disease progression accounts for another one-third. The remaining one-third is likely explained by other factors—such as age at onset and individual genetic and/or environmental factors influencing the progression of the degenerative process—and is likely to underlie the considerable heterogeneity in the clinical course of Parkinson's disease (Latourelle *et al.*, 2017). According to our calculation of the LDR based on pretreatment scores (natural OFF), we found that the true magnitude of the LDR ranges approximately between 60% and 65% of total motor benefit provided by levodopa. Most notably, we provide evidence that this is independent of disease duration. To date, the natural progression of motor disability in untreated Parkinson's disease has been calculated indirectly by using the pretreatment motor scores, postulating an identical rate of progression between treated and untreated subjects, and calculating the LDR by subtracting the overnight OFF score from the theoretical untreated score (Albin and Leventhal, 2017; Nagao *et al.*, 2019). Here, we found that the natural progression of motor disability in untreated patients does not parallel the progression of the overnight OFF disability on levodopa and thus it does not really decay over time, challenging the conceptualization that motor fluctuations emerge due to the progressive reduction in the LDR with relative increase in the SDR along with disease progression.

Our data expand the findings of a prospective study performed on treated Parkinson's disease patients during the first decade of treatment that estimated that the contribution of the LDR amounted to ~50% (Nagao *et al.*, 2019) and found that the magnitude of the LDR persists unchanged beyond the first decade. To the best of our knowledge, only one study has investigated the LDR in advanced Parkinson's disease by comparing 19 patients who underwent subthalamic nucleus deep brain stimulation and whose levodopa was discontinued for a period of 6 months versus 11 patients whose levodopa treatment had to be reintroduced postoperatively (Wider *et al.*, 2006). In line with the present results, the group of patients without levodopa showed greater worsening in the stimulation OFF state compared with the preoperative medication OFF state than the group ON levodopa. This finding supports the persistence of an LDR of a significant magnitude 15 years after onset, which was estimated to amount to 38% of the total levodopa effect (Wider *et al.*, 2006). We believe that this value is likely an underestimation of the effect of the LDR in advanced Parkinson's disease, mainly because postoperative UPDRS motor scores were assessed only 3 h after switching off the stimulation, which may not allow for an adequate washout of stimulation effects, as some effects (especially on axial features) may take several hours to a few days to wane as a consequence of pharmacodynamic changes within the basal ganglia network (Moro *et al.*, 2002). Accordingly, Wider *et al.* (2006) found that the LDR was not evident on axial signs but limited to bradykinesias and rigidity. In the advanced stages of Parkinson's disease, non-levodopa responsive symptoms are the main determinants of disability (Hely *et al.*, 2005). Considering the unique opportunity we had to observe the response to levodopa in patients with untreated Parkinson's disease even at advanced stages (as exemplified by the two paradigmatic video cases), we

provide evidence that the so-called 'non-levodopa-responsive' motor features may still have a robust and sustained response to levodopa even in advanced Parkinson's disease. We believe that this effect of levodopa on axial features—such as postural instability and frequent falls—has been underestimated to date because it may be less prominent when Parkinson's disease patients are treated and fluctuating (Kempster *et al.*, 2007; Evans *et al.*, 2011; Velseboer *et al.*, 2013), while we found it to be pronounced between the pretreatment condition and the motor performance at follow-up, not only in the ON state (reflecting the SDR) but also in the overnight OFF condition (reflecting the LDR). The strength of this effect is further emphasized by the significant difference in 'non-dopaminergic' scores in the OFF state even 2 years after the baseline assessment in the entire cohort. These data support the hypothesis that most axial features in Parkinson's disease may be—at least partly—responsive to long-term levodopa treatment, prompting the need for further research on this topic. Finally, we found that ~17% of untreated Parkinson's disease patients had clinically meaningful freezing of gait at baseline, which significantly improved after the initiation of long-term levodopa with a sustained long-duration response in the overnight OFF state at 1-year and 2-year follow-up. The present data on patients in the 'modern pre-levodopa era' would argue against the recent hypothesis of an increased likelihood of developing freezing of gait after the introduction of levodopa (Nonnekes *et al.*, 2020). Although it might be conceivable that freezing of gait presents with a lower prevalence in patients with longstanding untreated Parkinson's disease than in patients on levodopa treatment (Perez-Lloret *et al.*, 2014) due to greater severity of akinesia, which would preclude them from showing any freezing of gait, our prospective data support a favourable effect of long-term levodopa treatment on this axial motor symptom. We investigated the factors predicting the change between the motor performance at baseline (representing the 'natural' OFF state associated with the natural progression of Parkinson's disease) versus the overnight OFF at follow-up (which is a sort of 'combined' OFF reflecting the sum of the real disease-related OFF state plus the LDR to levodopa). Although we tried to take into account all major demographic and clinical variables available, our analysis could not identify any significant demographic or clinical predictor. This phenomenon may be explained by additional mechanisms that go beyond the mere storing capacity of residual nigrostriatal dopamine neurons, as is particularly evident in more advanced stages of the disease. Now, a critical question is: 'What are the plastic changes and the neuronal populations underlying the LDR to levodopa therapy even in the advanced stages of the disease, when the majority of nigral neurons have degenerated?' In this scenario, any therapy modulating the activity of this brain structure may influence the gap between the pretreatment state (natural OFF) and the overnight OFF after the initiation of daily levodopa intake. The mechanisms underlying the LDR are still up for debate (Anderson and Nutt, 2011; Albin and Leventhal, 2017; Nagao *et al.*, 2019). LDR has usually been associated with the early stages of the disease and it has been postulated that its underlying mechanisms occur at the postsynaptic level (Anderson and Nutt, 2011; Albin and Leventhal, 2017). In animal models, the LDR has been suggested to be associated with motor learning and pharmacodynamic changes in synaptic plasticity within the striatum (Anderson and Nutt, 2011; Albin and Leventhal, 2017). However, this model is not supported by the findings of the present study, which suggest more profound effects of levodopa treatment on Parkinson's disease motor symptoms. We hypothesize a two-step mechanism: an early LDR phase with a rapid onset (within hours) likely promoted by presynaptic mechanisms and a late LDR phase (requiring days to weeks to build-up), which is more probably associated with postsynaptic changes (Zappia *et al.*, 1999; Anderson and Nutt, 2011; Chou *et al.*, 2018). Our data suggest that postsynaptic mechanisms alone would not entirely explain the significant change we found between the natural OFF and the overnight OFF after just a few levodopa doses in untreated patients with advanced disease. Such a change would be better explained by presynaptic mechanisms. However, our hypothesis should be considered with caution due to the limited data available 24 h after the first-ever levodopa intake and needs further study to be confirmed. Although our multivariate analysis did not identify significant predictors of the LDR among demographic and clinical variables at baseline, it is worth mentioning that we found a strong correlation between the motor response at the first-ever levodopa challenge and the mean UPDRS-III score at overnight OFF at follow-up. This relationship between the first-ever SDR and the LDR at follow-up can be detected 24 h after the initiation of levodopa treatment and it is clear at 1-year follow-up, when patients' motor fluctuations are characterized by an overnight OFF state that closely resembles the ON state reached after the first-ever levodopa intake. Considering that receptor sensitization and other postsynaptic changes need longer periods (days to weeks) to occur, our data suggest that levodopa might exert a beneficial 'priming' effect at presynaptic sites, which probably adds to the mechanisms postulated to occur at postsynaptic sites (Anderson and Nutt, 2011) and deserves further study.

Strengths and limitations

We used a cross-sectional study design in a small—albeit unique—patient population to estimate the progression of motor symptoms both at baseline (natural course) and at follow-up (daily practice course). Although this methodological approach is clearly less robust than a prospective case-control observational study of patients left untreated for years to assess the natural progression of the disease, it is obvious that the more robust alternative is ethically unacceptable. Nonetheless, the study design is also prospective and serial measurements at 12 and 24 months showed substantial consistency in the association between disease duration and clinical rating, which enables us to argue in favour of a change

in the trend of progression of motor disability due to the LDR. Furthermore, compared to previous studies investigating the natural history of Parkinson's disease in the pre-levodopa era, we used the clinical scale that is currently considered to be the most accurate to measure disease progression (Parashos *et al.*, 2014). The main strength of this study is the assessment of a community-based cohort of untreated Parkinson's disease patients at a relatively advanced stage of disease at the time of diagnosis and initiation of levodopa therapy (compared to similar studies in Western countries) (Marras *et al.*, 2002; Evans *et al.*, 2011; Velseboer *et al.*, 2013; Cilia *et al.*, 2014). To minimize any inclusion or assessment bias and thus maximize the reliability of data, we decided to include only patients assessed by the same movement disorders specialist over the whole follow-up period, excluding patients assessed by multiple assessors. Although this approach provided a homogeneous assessment of all patients at all timepoints, we acknowledge that the lack of blinding may be prone to some form of bias *per se*.

In conclusion, although levodopa therapy is associated with motor fluctuations, we provide compelling evidence that OFF state disability is invariably less severe than at baseline after the initiation of levodopa therapy, even in the more advanced stages of the disease. Notably, response to levodopa included axial signs that are generally considered non-levodopa-responsive, including postural instability and falls. Our findings strongly discourage researchers from the use of the overnight OFF (or 'practical OFF' state) UPDRS-III score as primary outcome measure for the assessment of the progression of neurodegeneration in randomized clinical trials on disease-modifying strategies. Nonetheless, medication OFF motor score may still remain a valid clinical biomarker of disease progression in clinical trials whose design includes an adequate period of levodopa washout, which is still to be clearly identified and likely to last more than 4 weeks (Olanow, 2015; Leal Rato *et al.*, 2020). Further research is needed to identify the mechanisms underlying the LDR and the neuronal populations directly involved in this phenomenon when the majority of nigral neurons have degenerated. Taken as a whole, the present data suggest that the relative contribution of the LDR to total motor benefit provided by levodopa is greater than what was recorded in previous models, especially at advanced Parkinson's disease stages, and support the rationale of its early initiation (Espay, 2019; de Bie *et al.*, 2020; Leal Rato *et al.*, 2020). Understanding the mechanisms of the LDR may help to develop therapeutic strategies increasing its magnitude to improve patient quality of life. Finally, these data further emphasize the need to increase the accessibility to levodopa in low income countries, where it is still greatly limited (Cilia *et al.*, 2014, 2017; Mokaya *et al.*, 2016; Okubadejo *et al.*, 2018; Lim *et al.*, 2019), since its complex pharmacodynamic effect delays the progression of motor disability by acting through the LDR.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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