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A new, quantitative rating scale for dyskinesia in non-human primates

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Abstract

The aim of this study was to develop a quantitative scale to assess levodopa-induced dyskinesias (LID) in non-human primates using a video-based scoring system (Quantitative Dyskinesia Scale, QDS). Six macaques with stable parkinsonism and LID were used for tests of the new QDS, in comparison with our current standardized scale (Drug Related Side-effects, DRS), which provides a classic subjective measurement of dyskinesia. QDS scoring is based on systematic movement counts in time frames, using videotape recordings. For both scales, body segments scored included each extremity, trunk, neck and face, and raters were blinded to L-dopa treatments. Comparisons of the two scales revealed that their scores are highly correlated and parallel to the L-dopa pharmacokinetic profile, although the QDS provided significantly more quantifiable measurements. This remained the case after separating animals into groups of mild and severe dyskinesias. Inter-rater reliability for application of the QDS was confirmed with scores obtained by three examiners. We conclude that the QDS is a quantitative tool for reliably scoring LID in parkinsonian monkeys at all levels of severity of dyskinesia. The application of this new standard for scoring LID in primates will allow for more precise measurements of the effects of experimental treatments and improve the quality of results obtained in translational studies.

Keywords

non-human primate; dyskinesia; L-dopa; scale; MPTP

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Introduction

The development of abnormal involuntary movements, namely levodopa-induced dyskinesias (LID), is a common motor complication in Parkinson's disease (PD) that results from chronic dopamine replacement therapy (Marsden, 1982; Obeso *et al.*, 2007; Schapira *et al.*, 2009; Potts *et al.*, 2013). Dyskinesias can be very disabling, and although some therapeutic strategies have improved their management, none offers an optimal treatment for the majority of complicated PD patients (Obeso *et al.*, 2000; Chapuis *et al.*, 2005; Hametner *et al.*, 2010; Khan, 2012; Huot *et al.*, 2013). Therefore, the study of dyskinesia in animal models of PD is critical for understanding the mechanisms behind its generation, in order to develop new and more effective therapies (Duty & Jenner, 2011; Iderberg *et al.*, 2012; Morin *et al.*, 2013). In particular, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated non-human primate is a valuable model of PD for studying dyskinesias, because of the close resemblance of the involuntary movements in these animals to their appearance in patients (Clarke *et al.*, 1987; Crossman, 1987; Schneider, 1989; Collier *et al.*, 2003; Jenner, 2003b; Morin *et al.*, 2013).

A variety of methods for evaluating dyskinesia in monkeys is reported in the literature; however, their subjectivity and/or lack of validation have precluded the establishment of a standardized measurement tool (Petzinger *et al.*, 2001; Tan *et al.*, 2002; Fox *et al.*, 2012). While some of the current scales may provide a valid measure of dyskinesia, their qualitative nature limits their sensitivity and discriminative power in animal tests that depend on more objective assessment than a qualifying score from mild to severe given by the examiner. More significantly, these issues become problematic for a thorough evaluation of new antidyskinetic treatments that may have a small effect size. Hence, a quantitative scale is needed to provide a standardized method for measuring dyskinesia. The number of methods for evaluating dyskinesia quantitatively in NHPs is limited and those that are currently available focus on the time spent in hyperactivity, overall locomotion, beam brakes etc., which actually lack of specificity for involuntary choreodystonic movements (Pearce *et al.*, 1995; Chassain *et al.*, 2001; Kuoppamaki *et al.*, 2007; Johnston *et al.*, 2010a; Saiki *et al.*, 2010). Thus, there remains a need for a scale that directly and quantifiably measures truly dyskinetic movements, and it is important that such a scale be simple yet comprehensive, in order to increase the sensitivity for detecting small variations in dyskinesias. Here, we tested a new dyskinesia rating scale for parkinsonian monkeys that uses a video-based scoring system to yield quantitative data. This “Quantitative Dyskinesia Scale” (QDS) proved highly sensitive and reliable for monkeys exhibiting a range of LID severity.

Methods

Subjects

Six adult macaque monkeys (3 *Macaca fascicularis*, cynomolgus; 3 *Macaca mulatta*, rhesus) with moderate to severe bilateral parkinsonism were used (Table 1). Monkeys were rendered parkinsonian by repeated systemic injections of MPTP, as previously reported (Cao *et al.*, 2007). After stabilization of parkinsonian motor disability, all monkeys received oral levodopa [(L-dopa), 25–100mg; carbidopa/levodopa (Sinemet® 25/100)] 1–4 times

daily to induce dyskinesia. Oral L-dopa doses were determined on an individual animal basis according to the observed improvement of parkinsonian motor disability (“on” response). Animals were kept in controlled housing conditions with constant temperature, relative humidity, and a 12 hour light/dark cycle. Animals had free access to food, fresh fruit supplements, and water. All procedures were approved by the Institutional Animal Care and Use Committee.

Acute L-dopa tests

On test days, dyskinesias were induced acutely by subcutaneous (s.c.) injection of L-dopa (levodopa methyl ester plus 25% benserazide, dissolved in saline). Four L-dopa doses were tested, with each dose being repeated once for a total of 8 tests in each animal. L-dopa test doses were predetermined on the basis of reproducibility of motor responses with dyskinesias in each monkey, and the individual doses of L-dopa injections were kept constant throughout the tests. L-dopa doses were selected individually starting with the dose that induced clear reversal of parkinsonian symptoms and reproducible mild dyskinesias. Although not all animals received the same L-dopa doses because their behavioral responses differed (see Table 1 for L-dopa dose ranges used for each animal), the step between doses was the same among all animals. Specifically, for all animals, regardless of the starting dose (from 25 to 100 mg s.c.), there was a 25 mg difference between doses 1 and 2, 2 and 3, and 3 and 4. All tests were performed after overnight fasting and withholding Sinemet® treatment. Examiners remained blind to L-dopa doses until study completion.

Qualitative scoring

Parkinsonian motor disability score (MDS) and LID were assessed by trained examiners using a standardized motor disability scale for MPTP-treated non-human primates (Papa & Chase, 1996), which is similar to the Unified Parkinson's Disease Rating Scale (UPDRS) used for PD patients. Part I of the scale rates items associated with bradykinesia and tremor that yield the score of parkinsonian motor disability. In Part II of the scale (Drug-Related Side Effects, DRS), dyskinesias were rated qualitatively by scoring separately each body segment (face, neck, trunk, and right and left upper and lower extremities) from 0 to 3, with a total possible score of 21. Any abnormal involuntary movement such as twisting or swinging of the respective body part (e.g. wrist, shoulder, ankle, hip, trunk, tongue, jaw etc.) was deemed a dyskinetic movement. As with classic scales such as the DRS, no distinction was made between choreic and dystonic dyskinesias in the QDS (all dyskinesia types were counted). Of note, primate L-dopa induced dyskinesias are mostly mixed in nature, namely of choreodystonic type. The degree of severity of a given dyskinesia was determined according to the intensity of the abnormal movements taking into consideration its impact on volitional movement. Dyskinesias (choreodystonic involuntary movement) in each body segment were rated according to the severity/intensity and the frequency of the movement as follows: 0 = absent, 1 = mild and infrequent, 1.5 = mild and occurring frequently, 2 = moderate and occurring frequently, 2.5 = moderate and occurring most of the time (i.e. some pauses), 3 = severe and occurring constantly. Usually, dyskinesias are not developed in all body segments and their intensity varies according to the L-dopa dose. Note that additional scoring increments are defined here beyond what is found in the original publication (Papa & Chase, 1996); however, these scores are in keeping with the originally established wide

ranges and therefore do not represent any modification to the original scale. Likewise, the aim was to compare the two scales based on how they are or would be used in current studies, which entails real time qualitative scoring with the DRS versus offline scoring with the QDS. Thus, for DRS scores, live evaluations of the animals in their home cages were performed during the first 2–5 minutes of each scoring interval while the animals were simultaneously filmed for deferred scoring with the new scale (QDS). Animals were evaluated starting at baseline, 30 minutes post-injection and every 20 minutes thereafter until the MDS reached 50% of the baseline score (i.e. “off” state, before anti-parkinsonian drug treatment) or until dyskinesias were no longer present (typically after 70–150 minutes, depending on the L-dopa dose).

Quantitative scoring

In conjunction with DRS scoring, video recordings were taken for 2 minutes at the beginning of each scoring interval for later analysis using the new, quantitative dyskinesia scale (QDS). As with the DRS scale, any abnormal involuntary movement such as twisting or swinging of the respective body part was deemed a dyskinetic movement. However, rather than subjectively determining the degree of severity and frequency of dyskinesias during the 2 minute scoring interval, each observed dyskinetic movement that occurred during the 2 minutes was counted for each body part (e.g. every single twist of the left wrist counted as 1 dyskinetic movement in the left upper extremity). Specifically, the QDS entails counting the number of dyskinetic movements (as noted above) in each body segment in 20-second frames for the entire 2-minute video clip. For some animals, this required playing the videos in slow motion so that no movements were missed. Note that stereotypical movements such as lip smacking, turning or cage licking were not included in the scoring with either scale because the assumption that these primate behaviors are analogous to LID in patients remains controversial. The number of dyskinetic movements counted in each body segment was added to obtain a total number for each 20-second frame, and the totals of all 20-second frames of the 2 minute videotape were added to give a single dyskinesia score for each post-injection interval. Independently of one another, three blinded raters scored the same videos (the 50 minute interval after one L-dopa dosing in each monkey) in order to test the inter-rater reliability of the QDS.

Data analysis

Parameters of dyskinesia (total dyskinesia score as the sum of dyskinesias from all post-injection intervals, and peak-dose dyskinesia score taken at the 50 minute interval) were analyzed to validate the QDS and to determine its sensitivity for detecting a dose-response effect. Parametric tests were used since dyskinesia scores were graded within wide ranges using non-integer values; thus, data composed non-categorical variables. To compare dyskinesia scores between L-dopa doses, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. For direct comparison of the QDS and DRS scales, regression lines were generated for dyskinesia parameters using multiple linear regressions. To determine the value of the QDS for grading different degrees of dyskinesia, animals were grouped into two categories based on the severity of dyskinesia (mild, M, or moderate/severe, S). Inter-rater reliability was determined by one-way ANOVA of independent QDS

scoring of the same videos by three examiners (one video per animal). P-values equal to or less than 0.05 were considered significant.

Results

Validity of the QDS

To determine whether the QDS is a valid measure of dyskinesia, data obtained with the two scales were compared for dose 2 of L-dopa tests. Peaking at approximately 50 minutes post-injection and returning to baseline at the same time, data from the two scales followed the same time course of LID, which is demonstrated by the overlap of the two curves (Figure 1A). Furthermore, the time course of LID measurements using the QDS parallels changes in plasma L-dopa levels. Previous pharmacokinetic studies in primates (Cao *et al.*, 2007; Johnston *et al.*, 2010a) have shown that s.c. administration of L-dopa produces peak plasma levels between 45 and 60 min after injection. To confirm the parallel between the QDS and DRS demonstrated by the time course data, averages of scores from duplicate tests for each of the four L-dopa doses were analyzed. Total and peak dyskinesia scores were highly correlated between the two scales (Figure 1B and C, $R^2 = 0.88$ and 0.72 , respectively). Altogether, these data indicate that the QDS is a valid tool for measuring LID.

Sensitivity of the QDS

In addition to accurately measuring LID, it is important to determine the sensitivity of this new scale in order to assess its advantages compared to the classic dyskinesia rating scales. Hence, for the QDS and DRS scale, the L-dopa dose-response effects were analyzed. Scores generated from both scales gave rise to clear dose-response relationships for both total and peak dyskinesias (Figure 2). However, the QDS yielded a significant difference between L-dopa doses one and three, whereas the DRS scale did not detect such a difference. For L-dopa dose 1 and 3, mean $\pm$ SEM QDS total scores were $24.8 (\pm 10.5)$ and $97.6 (\pm 11.9)$, $p = 0.033$, respectively, but DRS total scores were $3.8 (\pm 1.6)$ and $15.0 (\pm 8.0)$, $p = 0.138$. QDS peak scores for the same L-dopa doses were $10.0 (\pm 3.8)$ and $30.6 (\pm 4.3)$, $p = 0.022$, but DRS peak scores were $1.4 (0.5)$ and $4.1 (\pm 0.8)$, $p = 0.115$. Therefore, the QDS is more sensitive than the DRS scale at detecting minor, yet significant, differences in LID.

Data thus far have demonstrated the validity and higher sensitivity of the QDS at measuring LID. To investigate further the sensitivity of the QDS, monkeys were split into two groups based on the severity of their dyskinesias (mild, $n = 3$ and moderate/severe, $n = 3$). Dyskinesia severity was determined by considering the intensity and frequency of dyskinesias as well as the number of body parts affected. Severely dyskinetic animals typically had dyskinesias in multiple body parts, which were moderate to severe in intensity and occurred at least 50% of the time (on average, L-dopa dose 1 produced DRS scores of 6.6 ± 2.1 total dyskinesias and 2.4 ± 0.7 peak dyskinesias). Mildly dyskinetic animals typically had no more than two or three body parts affected (e.g. lower extremities and tongue) with dyskinesias that were less intense and usually occurred less than 50% of the time (on average, L-dopa dose 1 produced DRS scores of 1.0 ± 0.5 total dyskinesias and 0.5 ± 0.2 peak dyskinesias). In each group, the relationship between L-dopa dose and total or peak dyskinesia scores from the QDS and DRS scale was analyzed by multiple linear

regression to compare the slopes between the two scales. In both mild and severe LID groups, the QDS yielded higher dyskinesia scores (both total and peak scores) with rising L-dopa doses than the DRS scale, due to the limited range of the latter scale. Moreover, significant slope differences between the two scales were found in both groups of mildly and severely dyskinetic animals (Figure 3) indicating clearer distinction between L-dopa doses by the QDS. Thus, these results underscore the quantitative nature of the QDS relative to the DRS scale, and reveal its improved sensitivity regardless of dyskinesia severity.

Reliability of the QDS

Using the QDS, all three raters provided similar dyskinesia scores for all but one monkey ($n = 6$; Figure 4). In the discrepant case, only one of the rater's score differed from the other two, whose scores were in agreement with each other. Thus, regardless of this isolated discrepancy, the scale's reliability was demonstrated clearly as scores given by the three raters were not significantly different from one another ($p = 0.83$).

Discussion

Here we have described a new, quantitative tool for assessing dyskinesias in the non-human primate model of Parkinson's disease. As this is a newly developed scale, the goal of this study was to validate the QDS for accurately and quantitatively measuring dyskinesias. This was done by comparing data to that generated using our existing, qualitative scale (DRS) (Papa & Chase, 1996; Cao *et al.*, 2007). First, QDS scores correlated strongly with scores from the DRS scale within the time course of LID and with plasma L-dopa levels (Cao *et al.*, 2007). Second, the QDS was significantly more quantitative than the DRS scale demonstrating its enhanced sensitivity. Finally, the QDS proved to be reliable for yielding consistent dyskinesia data among different raters.

We determined the QDS to be a valid tool for measuring dyskinesia considering its direct overlap with the DRS scale as well as its correspondence with previously published pharmacokinetic data for L-dopa. Although the plasma L-dopa levels previously reported by Cao *et al.* (Cao *et al.*, 2007) are from a different group of animals than those used in the present study, both studies included rhesus macaques and LID were modeled following the same MPTP and L-dopa administration protocols. Therefore, the pharmacokinetics L-dopa are not expected to be different between the two groups of animals. The significant difference in scores between the two scales highlights the quantitative nature of the QDS, which is achieved by counting every single observed dyskinetic movement. At first glance it may seem that the QDS just provides higher numbers, but its ability to rate dyskinesias in a wider range results in higher discriminating power for small changes. As expected, the QDS yielded a clear dose-response effect of L-dopa on dyskinesias (Jenner, 2003a; Kuoppamaki *et al.*, 2007), detecting differences between doses that did not reach significance with the DRS scale. It is likely that the difference between doses were too small for the DRS to reach significance as a result of the inter-animal variability within a relatively limited range of scores (the maximum possible score for DRS is 21). The discrepancy between the two scales underscores the higher sensitivity of the QDS for detecting small changes in dyskinesias that might not be detectable by qualitative scales. Notably, the QDS detected an L-dopa dose-

response in both mildly and severely dyskinetic monkeys, providing more quantitative data than the DRS scale in both groups. This is important considering that for many studies there could be a wide range of LID severity among animals, which may be difficult to detect using qualitative measures. Further, because of its higher discriminating power the QDS has an increased sensitivity for assessing homogeneity of dyskinesia across animals; thus, the QDS may also be useful as an inclusion criteria tool to reduce the experimental variability of a study and provide increased power while minimizing the number of animals (Potts *et al.*, 2013).

The present results also demonstrate that setting distinct time-frames for counting dyskinetic movements in all body parts and excluding stereotypical behaviors provided a standardized measurement that could be reproduced among raters. Thus, the high inter-rater reliability is a valuable feature of the QDS. It is important to note that the purpose of this study was not to provide a scale elaborating on the definition of dyskinetic movements in this model, but to provide a standardized method for quantitatively measuring these movements that may be useful in a wide range of studies. Thus, it is critical that examiners are well trained in recognizing dyskinetic movements in order to properly apply the QDS.

In keeping with the goal of therapeutic studies in PD / LID, the QDS is a reliable tool for detecting small changes in the severity of monkey's dyskinesias, which may be useful in determining the therapeutic index of experimental drugs. Thus, though qualitative scales may provide useful information regarding substantial changes in dyskinesias, a scale that accurately measures small differences is advantageous for full assessment of drug efficacy and therapeutic window in translational pre-clinical studies. Furthermore, this quantitative scale could be used to set a numeric “effect threshold” that must be reached to justify further development in clinical trials. Such a threshold may help reduce the number of translatable failures due to exaggerated efficacy data obtained from a limited-range scale in preclinical tests.

The QDS represents a simple, easily quantifiable measure of dyskinesias without the need for special test cages, specific cage set-ups or quantification software as required for other existing quantitative scales (Chassain *et al.*, 2001; Jarraya *et al.*, 2009; Johnston *et al.*, 2010a). For example, the approach employed by Jarraya and colleagues relies on video analysis software to detect and count dyskinetic movements. While this method is undoubtedly quantitative and eliminates the dependence on reliable scorers that is maintained with the QDS, there remains the possibility of detecting false positives and/or excluding subtle dyskinetic movements since the software is programmed to detect specific, pre-programmed movements. Furthermore, the QDS evaluates the observed dyskinetic movement(s), providing a direct measure of LID compared to indirect measures such as overall locomotion, interference with “normal” activity, or quality of “on” time that is commonly used for evaluating dyskinesias (Pearce *et al.*, 1995; Petzinger *et al.*, 2001; Tan *et al.*, 2002; Johnston *et al.*, 2010a, b; Fox *et al.*, 2012). Measuring the relative amount of hyperactivity may be a reliable indicator of response to dopaminergic stimulation but it is not a true measure of dyskinesia. Additionally, scales based on interference with normal activity introduce the subjective interpretation of an animal's intention to move in order to evaluate interference with activities of daily living. Even though severe dyskinesias may

significantly affect normal ambulation and mobility, this type of scoring has limited sensitivity for finely detecting dyskinesia severity.

In conclusion, we have presented a standardized method for quantifying dyskinesia in non-human primates. This scale is simplistic in nature yet sensitive for reliably detecting a wide range of dyskinesia severity. The QDS was validated with L-dopa, which is the primary and standard agent for eliciting dyskinesias in parkinsonian animals. As this new scale is valid and highly sensitive for detecting changes in dyskinesias with L-dopa dose-response curves, it is expected that the QDS would be equally sensitive for detecting variability in LID by co-administration of anti-dyskinetic agents. Thus, the present data support the integration of this new scale in studies evaluating potential therapies for LID in non-human primate models of Parkinson's disease.

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Figure 1. Correlation of dyskinesia scores between QDS and DRS scales

There was a significant correlation between the two scales in the entire group of animals ($n = 6$). (A) Example of the time course of dyskinesia following L-dopa dose 2 administration, demonstrating the correlation of the two scales. Scores were taken at 0, 30, 50, 70, 90, 110, 130 and 150 minutes post-injection. The solid black line with the filled gray shaded area represents the mean $\pm$ SEM for the DRS scale, while the thick dashed line with the dotted curves bordering the hatched area represents the mean $\pm$ SEM for the QDS. (B) Total (sum of dyskinesia at all time intervals) and (C) peak-dose dyskinesia scores (50 min interval). Data points represent the mean of duplicate tests from each dose for each animal ($n = 24$). Linear regression analyses, R^2 for regression line, $p < 0.001$ for total and peak dyskinesia.

Figure 2. The QDS is more sensitive than the DRS scale at detecting dose-dependent changes in dyskinesia

A total of four L-dopa doses were tested twice, and the doses were predetermined for each monkey ($n = 6$). Both scales show a clear dose-response relationship. The QDS (A and B) was more sensitive than the DRS scale (C and D) at detecting minor differences between doses in both total (A and C) and peak (B and D) dyskinesias. One-way ANOVAs with Tukey's post-hoc tests. Bars represent the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ compared to dose 1. # $p < 0.05$ compared to dose 2.

Figure 3. The QDS is more quantitative than the DRS scale and maintains sensitivity regardless of dyskinesia severity

Monkeys were grouped according to severity of dyskinesia (mild or severe, $n = 3$ in both groups) and each group analyzed separately. There were significant differences between the dose-response relationships of the two scales for total (A and B) and peak-dose (C and D) dyskinesias in both mildly (A and C) and severely (B and D) dyskinetic monkeys. A total of four L-dopa doses were tested that were predetermined for each monkey. Multiple linear regressions comparing slopes of the best-fit lines. Data points represent the means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for significant differences between the slopes of the two lines.

Figure 4. Inter-rater reliability of the QDS

Three trained examiners scored the same videos (the 50-min interval from one L-dopa dosing in each of the 6 monkeys) independently of one another without knowledge of the dose or other raters' scores. Inter-rater reliability was confirmed by a lack of significant differences between the raters' scores. One-way ANOVA, $p = 0.83$.

Table 1

Demographics of parkinsonian macaques.

Monkey #	Species	Age (yrs.)	Gender	Weight (kg)	MDS-Off	MDS-On	DYSK	L-dopa Dose Range (mg) ^a
1	C	4	F	4.0	18	7	M	25–100
2	R	12	F	5.7	23	12.5	M	100–175
3	C	6	M	5.8	25	6	M	25–100
4	R	8	F	5.1	16	4.5	S	100–175
5	R	8	M	6.8	19.5	5	S	75–150
6	C	6	M	5.6	25	13	S	25–100

Cynomolgus (C) and Rhesus (R) macaques with various degrees of parkinsonism induced by systemic MPTP treatment. Animals were evaluated with the standardized motor disability scale for MPTP-treated monkeys (Papa 1996). MDS-Off = parkinsonian motor disability score in the off state. MDS-on = parkinsonian motor disability score at peak effect following s.c. injection of optimal L-dopa dose, which produced a minimum of approximately 50% reduction of motor symptoms with clear and consistent dyskinesias in repeated tests. MDS ranges from 0–39 (scores > 20 represent severe impairment). DYSK = dyskinesia severity; M = mild, S = severe.

^aL-dopa dose ranges (mg) for each animal were based on observations of prior responses to various L-dopa doses in order to determine the lowest dose that produced a reduction in parkinsonian symptoms and reproducible mild dyskinesia. The range for each animal was selected starting with the predetermined initial dose and increasing by 25 mg each higher dose. Additionally, on the basis of our knowledge of each animals' sensitivity to L-dopa, care was taken in the selection of each dose range to ensure that doses were neither too low nor too high in order to avoid either missing a range that would elicit dyskinesia or causing a dyskinetic response so severe that any dose-response effect would be masked by the saturated L-dopa response.