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Quantification of movement in normal and parkinsonian macaques using video analysis

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Abstract

Background: Quantification of spontaneous animal movement can be achieved using analysis of video recordings of the animals. Previous reports of video-based methods are based on outdated computer platforms or require the use of specialized equipment.

New method: We developed a video analysis algorithm to quantify movement based on the commonly used MATLAB programming language. The algorithm is based on pixel differences between frames of video footage acquired with a standard video camera.

Results: The new algorithm was validated, analyzing the amount of movements made by monkeys undergoing treatment with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce parkinsonism. We compared the movement quantification generated by the new system of analysis with results obtained with a conventional infrared beam break counting system, a parkinsonism rating scale, and accelerometry-based motion quantification in three rhesus macaques. The information provided by our video analysis method was consistent with that obtained with the first two methods, and more detailed than the third.

Comparison with existing methods: The new method can replace other methods to quantify movement. Although other video analysis methods have been described, some have since been deprecated, or involve the use of specialized hardware. The new method provides a straightforward and fast approach of analyzing the amount of movement in caged experimental animals, using conventional off-the-shelf equipment and moderate computing resources.

Conclusions: This video analysis method provides an affordable, open access platform to quantify animal movement.

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Keywords

Video-based movement analysis; nonhuman primates; movement quantification; parkinsonism

Introduction

Rhesus macaques treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are used as a phenotypically reliable non-human primate (NHP) model of Parkinson's disease (Blesa et al., 2012). The animals develop bradykinesia, rigidity, freezing and other parkinsonian signs. A variety of tests can be used to assess the animal's degree of parkinsonism. These tests commonly include rating on a parkinsonism scale (Imbert et al., 2000). Other methods seek to quantify the amount of movement produced by the monkey. The latter require, in many cases, the use of specialized equipment, such as enclosures equipped with infrared beams, or accelerometers (e.g., Emborg, 2004; Wichmann et al., 2001).

Activity levels have also been assessed using computer-based analyses of video recordings of NHPs (Brouillet et al., 1995; Campos-Romo et al., 2009; Chassain et al., 2001; Hashimoto et al., 1999; Togasaki et al., 2005). However, in many cases the software is based on outdated computer systems or is proprietary, and the video analysis requires specialized equipment. Here we describe a new video analysis algorithm to quantify the movements of NHPs based on the commonly used MATLAB (MathWorks, Natick, MA) programming language. This method does not require additional equipment, other than a standard video camera.

Methods

Animals and MPTP treatment

These studies were done in accordance with the "Guide for the Care and Use of Laboratory Animals" (Garber et al., 2011), and the United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 2015), and were approved by the Animal Care and Use Committee and the Biosafety Committee of Emory University. We used 3 rhesus monkeys (2 males, 1 female, monkeys B, H and N; approximately 4 years old at the beginning of the study). The monkeys were obtained from the colony at the Yerkes National Primate Research Center, were pair-housed with other monkeys, had free access to food and water, and received vegetables and fruit daily. Monkeys were trained to be handled by the investigators and acclimated to the laboratory environment. Monkeys B and H received MPTP (Natland International, Morrisville, NC; 0.2–0.8 mg/kg, i. m.) once weekly to induce parkinsonism. Monkey B received a total of 2.4 mg/kg of MPTP for 6 weeks, while monkey H received 7.85 mg/kg over the course of 20 weeks. Monkey N did not receive MPTP.

Assessment of parkinsonism using traditional methods

Before starting the MPTP treatment, the monkeys were habituated to the observation cage (28×24×35 inches). This cage was equipped with eight infrared beams and corresponding

reflectors, mounted in two rows of four beam/reflector pairs, front-to-back and side-to-side of the cage. The inside of the cage was devoid of movable objects (such as hanging or rolling toys). The timing of the occurrence of beam crossings was collected to computer disk, thus allowing us to quantify the number of times the beams were broken as a measure of the animal's spontaneous movement. The habituation period consisted of 3 daily sessions of 30 min each. No measurements were obtained during these sessions. Subsequent baseline observations consisted of 20-min sessions of cage observations, spaced by 1 to 7 days. In each session, the first 5 min were used as a daily habituation period, and the movement data was collected during the next 15 min. We collected 9 and 8 sessions in the normal state for monkey H and B, respectively. Data from Monkey N is based on one observation session in the normal state (see below).

During the MPTP treatment, we also evaluated parkinsonian signs while the monkeys were in the observation cage. For this, we used a parkinsonism rating scale (PRS) (Devergnas et al., 2014; Galvan et al., 2014; Wichmann et al., 2001) that included scores for bradykinesia, arm and leg akinesia, limb and trunk posture, action tremor, finger dexterity, balance, and freezing; each item was scored 0–3 (absent to severe), for a maximum of 27 points.

To determine the effects of MPTP on the amount of movement, the baseline for each monkey was defined as the average of the daily infrared beam break counts (or median *m* scores for the video analysis, see below) across all sessions before the first MPTP injection.

To compare the data obtained with the video analysis with the data provided by accelerometry, an accelerometer (Actical, Respironics, Murrysville, PA) was mounted on a plastic collar worn by the third monkey (monkey N). The proprietary accelerometer software provides arbitrary counts of the amount of movement registered by the device every second. The data collected by the accelerometer was synchronized with the video analysis. To further identify how the movements of various body parts contributed to the measurements obtained with the video analysis or the accelerometer, we used an additional computer-assisted method to quantify movement. For this, a researcher coded the movements made by the monkey by pressing different keys on a keyboard, each key indicating movements of individual body parts (such as limbs, head or torso, Wichmann et al., 2001).

Assessment of parkinsonism using video analysis

Video acquisition—Video recordings were obtained for each of the 15 min session spent by the monkey in the observation cage. We used a video camera (models VIXIA HRF600 or HFM30, Canon), acquiring video at 30 fps in RGB. The camera was mounted on a tripod, 1.06m off the ground, 1.9 m away from the cage, the lens pointing perpendicularly to the center of the front panel of the cage. The cage and camera were always positioned in the same location in the test room. To avoid flicker in the video signals, the room was illuminated with an LED light. The video recordings were saved as MPEG4 files.

Calculation of movement scores—We created a MATLAB algorithm to analyze the acquired videos (available at <https://github.com/DrCaiola>). The analysis is based on an evaluation of frame-to-frame pixel differences, based on the assumption that the primary picture element contributing to differences between video frames is animal movement, as

long as the observation environment and illumination remains stable. Although we compared every frame (in sequential order) to achieve high temporal resolution, the code allows for temporal downsampling, by using fewer frames (for example 1 of every 10 frames). In addition, the observation area (in our case, the cage), was empty of moving objects that could interfere with the quantification of the animal movements.

The user defined the segment of the video to be analyzed to begin at the one-minute mark and continued for either 14 (Monkey B) or 15 (Monkey H and Monkey N) minutes in length to avoid any interference from the experimentalist entering/leaving the room. The same time segments were used for the analysis based on the infrared beam quantification.

The MATLAB script displays the first frame of the selected segment and prompts the user to draw a rectangle around the region of interest (ROI). In this case, we always selected the entire cage area, to avoid artifacts from areas around the cage (blue rectangle, Fig. 1). For each frame of the video, the ROI is extracted, and the resulting image converted to grayscale, shrunk from the original (whole frame) resolution of 1280 by 720 pixels to a resolution of 360 by 240 pixels, and pixel values are normalized to the intensity range in the ROI to reduce the potential effects of illumination differences and video noise on the analysis.

For every pixel (x, y) in frame k , $f_k(x, y)$, we calculate the absolute normalized luminance difference, d , from the previous frame: $d_k(x, y) = |f_k(x, y) - f_{k-1}(x, y)|$. To control for noise, we set pixel changes of less than 10% of the previous frame to 0. Using the matrix of d -values, three movement values were defined for each pair of frames: the m , m_{sign} , and m_z scores.

The m score is the mean of d in the ROI, denoted here as $m = \overline{d(ROI)}$ with the bar representing mean.

The m_{sign} score compares the d values belonging to pixels within the ROI to those outside of it. The pixels outside of the ROI are considered to be the same (given that no change occurred in the environment), but, of course, differ slightly from frame to frame depending on video noise and shifts in illumination. The m_{sign} score is defined as

$$m_{sign} = \frac{\# \text{ of pixels}(d(ROI) > \overline{d(-ROI)} + 2 \text{std}(d(-ROI)))}{\# \text{ of pixels}(ROI)}$$

with $-ROI$ denoting the region outside of the ROI and std representing standard deviation, thus representing the fraction of pixels whose difference values d surpassed those of the mean of the pixels outside of the ROI by more than 2 standard deviations.

The m_z score also compares pixel differences between the ROI pixels and those outside of the ROI. In this case, the d values are z-scored using the pixels outside of the ROI:

$$m_z = \text{mean} \left(\frac{|d(ROI) - \overline{d(-ROI)}|}{\text{std}(d(-ROI))} \right).$$

The three scores (m , m_{sign} , m_z) are vectors of (frame – 1) values each. To smooth the score vectors, they were median filtered with a bin length of 3 points, thus retaining the high temporal resolution of the analysis. Median values of the entire data stream from a given observation session were subsequently graphed to represent the animal's movement before, during, and after MPTP treatment (Fig. 2).

The m score corresponds to changes in the average intensity in the ROI, thereby providing a measure of the overall amount of movement. We found this measurement score accurate for all our needs, however, if needed, the m_{sign} and m_z can also be computed by the algorithm.

A Mann-Whitney U test was used to compare the observation sessions before and after MPTP treatment (Fig. 2) for both monkey H and B with our significance level set by $\alpha = 0.05$.

Evaluation of background and lighting conditions on the video analysis

measures.—To compare the information provided by the three video analysis scores, and to study how different light conditions and different backgrounds could influence the results of the analysis, we used a plush monkey balanced on top of a rotator plate, which moved at moderate speed (see Supplemental figure 1A). The toy and plate provided a repetitive and standardized movement that served as control as we changed other parameters of video acquisition. The videos were collected in the same room where the behavioral observations of the monkeys were conducted. Four videos were acquired under the following conditions: plain white background with full illumination of the room (~780 lux), plain white background with partial illumination (~280 lux), plain white background with no light (0 lux), patterned background with full illumination (See Supplemental figure 1B). The rotator plate was started, and videos were collected for 5 minutes in each condition.

Results

Comparison of the video analysis scores and environmental conditions for video acquisition

The results of the analysis of control videos of a toy moving on an automated rotating plate are shown in Supplementary Fig. 1. Videos were acquired on different light conditions, and using a patterned background. All three video analysis scores (m , m_{sign} , and m_z) were similar across sessions, with no movement score being significantly affected by the different conditions. The only exception was the condition of no light, which consistently reduced all three scores. This data indicates that the video analysis algorithm is robust to detect movement under different conditions, with illumination as low as ~280 lux (in our setting).

Comparison of MPTP-induced parkinsonism assessed by traditional methods and video analysis

—MPTP treatment induced parkinsonian signs in both monkeys. Figure 2 shows the assessment of movement and parkinsonism in 2 monkeys during baseline and through the course of MPTP administration. The monkeys were evaluated for 24 and 9 weeks (monkey H and B respectively) after the start of MPTP treatment (indicated by a vertical dashed line in fig. 2). For both monkeys, the PRS values gradually increased with the MPTP treatment (Fig. 2A and 2E). The movements in the observation cage were

monitored by quantifying the number of times an infrared light beam was crossed (Fig. 2B, 2F). Before the start of the MPTP treatment, the amount of movement made by the monkeys, assessed by this method, was variable (monkey H: 1305.6 ± 594.37 , monkey B: 382.1 ± 162.6 , during a 15 min observation period, average of baseline sessions (sessions 1 to 10 for Monkey H and 1 to 8 for monkey B).

As expected, after the start of the MPTP treatment, the monkeys moved less, reflected in a reduction in the number of beam breaks, which reached a maximal 99.8% and 98.4% reduction from baseline (monkeys H and B respectively, Fig. 2B and 2F).

Fig. 2C and 2G show the results from the *m* score analysis of video recordings obtained during the same observation sessions. The median daily movement scores match closely the beam break measurements in individual sessions (compare Fig. 2B with Figs. 2C). The *m* scores closely follow the peaks and troughs observed in the beam break data. In addition, the reduction in the movement score matched the increase of the parkinsonism score. Both beam breaks and *m* scores measures dropped below the 25th percentile of the baseline values after the first (monkey H) or second (monkey B) administration of MPTP, but the change in the *m* score was more pronounced relative to baseline. The *m* scores obtained in all 24 sessions (Monkey H) and 9 sessions (monkey B) after MPTP the start of treatment were found to be significantly different than the baseline period ($p < .0001$ for Monkey H and $p = 0.0016$ for monkey B; Mann-Whitney U Test).

Fig. 2D and 2H show the correspondence between the reduction of *m* scores and beam break counts during the administration of MPTP. Both measures decreased in parallel as parkinsonism increased, as determined by the parkinsonism score. However, the *m* scores (blue symbols) were obviously more sensitive to the reduction in movement than either of the other measures.

Comparison of movement quantification with accelerometer and video

analysis—Accelerometers are commonly used to assess amount of movement in NHP experiments. In monkey N (only studied in the healthy state), we used an accelerometer mounted on the monkey's collar to quantify the amount of movement in the observation cage. We compared the results to the data acquired with a computer-assisted method in which a user quantifies the movements made by the animal by coding each movement with keyboard presses (see Methods), and to our video analysis method. As shown in Fig. 3, the accelerometer counts did not accurately reflect the movements produced by the monkey (as coded by the human observer). The accelerometer failed to identify movements of head, arms and legs when the monkey remained sitting in the same place (Fig. 3A red line and 3B, note for example, minutes 9 and 10). The video analysis, in contrast, detected more of the limb and head movements, in addition to trunk displacements (Fig 3B blue line and symbols).

Temporal resolution of the video analysis method—Although counting beam breaks provides an adequate quantification of movement across a 15-minute session, it does not accurately reflect small movements. The coarse sampling of beam break events allows the monkey's movements between beams to go undetected. In contrast, the video-derived

movement scores are calculated frame to frame, and thus allows for continuous or live movement tracking with the additional benefit of a much higher spatial resolution. An example video of Monkey N and its associated m score is shown in Video 1.

To further demonstrate the high temporal and spatial resolution of the video analysis method, Fig. 4 shows the comparison of the movement scores in a representative segment of 1 minute of data (Monkey H, session 24, minute 2). During this minute, the monkey remained in the same location in the cage (which resulted in only one beam break, not shown). The video analysis, instead, detected several movements, such as head-turning at second 10 and 37, chewing from second 23–32, hand movements from seconds 41–42, and a body turn at seconds 48–52. The m and m_{sign} scores (Fig. 4A and 4B) quantified these movements similarly. The m_z score (Fig. 4) relies on the standard deviation of the area outside the ROI, which results in abrupt rises and falls in the scoring.

Conclusions

This report describes a simple method to quantify movements, based on video recordings that can be easily obtained using a standard video camera, and without the need of specialized hardware such as accelerometers or other sensors. Furthermore, animals do not require training beyond habituation to the observation enclosure.

The information provided by the video analysis is generally comparable to that obtained by the infrared beam break counting method, and reliably reflects reduction of gross movements in the cage as the MPTP-induced parkinsonism develops. However, the video analysis method has several advantages over the beam break counting method. Our analysis shows that the video analysis is more sensitive than the infrared beam break method in the detection of subtle deficits in movements, particularly in the early phases of MPTP treatment. Further, while the spatial resolution provided by the infrared beams depends on the number and arrangement of beams in the testing area, the spatial resolution of the video analysis method is much higher, limited only by the pixel resolution of the videos. In addition, our video analysis method can provide a more continuous readout of the movements of the animal (as shown in fig. 4). Finally, with fewer equipment components within the monkey's reach, we avoid additional errors or equipment failure caused by the animal manipulating cables.

Our results also suggest that the video analysis provides a more accurate description of overall movements than the information provided by the use of collar-mounted accelerometers, especially when used on a single subject. This description can also be obtained by a human observer who codes and quantifies individual movements of the animals, but we have shown that the video analysis provides comparable information about the overall amount of movement. While much less time-consuming, the video analysis does not completely obviate human observations, because it does not provide information about movements of individual body parts (see also below).

The occurrence of tremor would likely be detected as an increase in the m score, and thus, be a possible confound for the identification of akinesia. Use of the light beam breaks would

lead to a similar problem. However, in reality, this does not present a major problem for behavioral assessments in the most commonly used NHP species (Rhesus monkeys), because significant tremor is rare in these animals. Further, if tremor is unusually present in a given animal, detection of these involuntary movements can be reduced by not using all frames of the video record for movement detection. Spacing the frames for analysis by 0.5–1 s would almost certainly avoid detection of tremor.

Similar to our method, previous reports of computer-based video analysis of monkey movements use frame-by-frame analyses to quantify displacement of the animal, based on the number of pixels that change from one frame to the next (Campos-Romo et al., 2009; Chassain et al., 2001; Hashimoto et al., 1999; Togasaki et al., 2005). Some of these methods require specialized cameras and proprietary software. In others, the software is based on outdated computer systems, or is no longer available. Our video analysis algorithm to quantify movement is based on the commonly used MATLAB programming language, and we have made it accessible through GitHub.

A limitation of our method is that it only detects movement in two dimensions. Movements forward and away from the camera may not be detected. Another consideration is that the algorithm will count all objects moving in the selected frame, for instance, objects hanging from the cage, or enrichment toys inside of the cage. This can be corrected by excluding these objects from the analysis frame (if the objects are placed in a fixed location), or by removing the objects from the cage before starting the video recording.

This method provides an affordable, open access platform to quantify movement in a non-human primate model of parkinsonism or other movement disorders. Due to its simplicity, this video analysis method can also easily be incorporated in other experimental conditions that require quantification of movements. Obvious improvements would include the analysis of movements of individual limbs (as has been published recently, Mathis et al., 2018). However, these methods would likely require a much greater involvement of the user, and would require a large amount of computing power/hardware.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Simple movement analysis utilizing a single stationary video camera
- Available code in MATLAB
- Method quantify movement with precision similar to infrared beam break counting
- Tracks full body movement better than collar-attached accelerometers
- The method can provide animal motion data of high temporal precision

Fig 1.

An example of ROI selection. Screenshot taken in the Matlab software during ROI selection. The blue rectangle flanked by the squares indicates the ROI selected for our analysis. We selected the entire cage, excluding objects around the cage.

Fig 2:

Assessment of movement before and during MPTP-induced parkinsonism, in monkey H (Fig. 2A-D) and monkey B (Fig. 2E-H). Sessions were separated by 1 to 7 days. A, E) Parkinsonism rating scale scores (PRS) assigned during MPTP treatment. B, F) Number of infrared light beam breaks generated by the monkey in the observation cage. The x-axis displays the number of sessions, where 11 and 9 indicate the first session during the MPTP treatment for monkeys H and B respectively. C, G) Movement score (m score) obtained using the video analysis. The medians for each session are indicated by red circles and line, while the zone between the 25th and 75th percentiles of the baseline values is denoted by the shaded yellow region between the dotted lines. The baseline period (session 1 to 10, monkey H, and sessions 1 to 8, monkey B) finishes at the vertical dashed line. D) Comparison of movement and beam breaks to the parkinsonism score. Blue symbols and lines represent median daily m scores while orange symbols and lines represent daily beam breaks.

Fig. 3: Movement detection using a collar mounted accelerometer and video analysis. A. Counts produced by a collar-mounted accelerometer during an 18 min observation epoch (red symbols and line, right y-axis) and video analysis m score of movements produced by the monkey during the same session (blue symbols and line, left y-axis). B. For the same session as shown in A, the lines indicate the number of movements of different body parts per minute, as recorded manually by a researcher using key presses to code each movement.

Fig 4: Comparison of frame-by-frame movement scores over a one-minute span during an observation session for monkey H (the minute corresponds to minute 2 in session 24 of Monkey H).