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# Differentiating early Parkinson's disease and multiple system atrophy with parkinsonism by saccade velocity profiles

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HIGHLIGHTS

- Patients with multiple system atrophy with parkinsonism (MSAP) showed low saccade peak velocity compared with normal subjects.
- The deceleration and acceleration periods of the velocity profile were prolonged in MSAP.
- Patients with Parkinson's disease showed normal acceleration and peak velocity but longer deceleration.

ABSTRACT

*Objective:* Patients with Parkinson's disease (PD) and multiple system atrophy both present predominantly with parkinsonism at early stages, whereas cerebellar symptoms are largely masked in multiple system atrophy with parkinsonism (MSAP). We sought to determine whether the velocity profiles of saccades could be used to differentiate between these two disorders, revealing the underlying basal ganglia and/or cerebellar dysfunction and brainstem pathology in these disorders.
*Methods:* Sixteen MSA-P patients, 63 PD patients, and 36 age-matched normal subjects performed the visually guided (VGS) and memory-guided saccade (MGS) tasks. Targets were presented at eccentricities of 5, 10, 20, and 30 degrees. The amplitude, peak velocity, and duration of saccades were compared among subject groups. Duration was further subdivided into acceleration and deceleration periods, corresponding to the times before and after peak velocity. These parameters correlated with the severity of Parkinsonism as assessed by the UPDRS motor score.
*Results:* Hypometria predominated in both PD and MSAP patients, whereas hypermetria, frequently noted in cerebellar ataxia, was rarely observed. Saccades in MSAP were characterized both by prolonged acceleration and deceleration periods with reduced peak velocity. In contrast, the velocity profile of PD patients was characterized mainly by the prolonged deceleration period. The changes observed in velocity profiles of MGS deteriorated with advancing severity of parkinsonism in MSAP and PD patients.
*Conclusion:* Saccade profiles provide useful information for differentiating between PD and MSAP at early stages. While the changes in velocity profiles may be explained by the cerebellar and brainstem pathology in MSAP, the changes in velocity profile in both PD and MSAP correlated significantly with increasing severity of Parkinsonism in both disorders, suggesting a link with striatonigral pathology.
*Significance:* The differential changes in saccade velocity profiles of MSAP and PD may be used as a measure indexing the progression of cerebellar and basal ganglia dysfunction as well as for assessing the functional improvement when clinical treatment becomes available.


## 1. Introduction

Multiple system atrophy is a neurological disorder, featuring both cerebellar and basal ganglia pathology, and thus can present

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either with cerebellar ataxia, parkinsonism, or both. Actually, clinical presentation of MSA with predominant cerebellar ataxia (MSAC) resembles that of spinocerebellar ataxia with pure cerebellar presentation (SCD), both of which are almost indistinguishable from each other at their early stages. On the other hand, MSAP with predominant Parkinsonism (MSAP) resembles Parkinson's disease (PD) at early stages, both presenting with parkinsonism. In due course, severe autonomic syndromes will manifest themselves both in MSAC and MSAP, unmistakably differentiating them from SCD and PD. In view of the recent advances in possible treatment measures of MSA (Mitsui et al., 2013), however, early diagnosis of MSAC and MSAP, by differentiating them from other neurological disorders showing cerebellar ataxia and parkinsonism, would be essential for modifying the disease course and reaching a better outcome for this devastating disorder through early intervention.

While we have addressed the differentiation of SCD and MSAC in our previous studies (Terao et al., 2016, 2017), distinguishing PD and MSAP is also sometimes difficult at their initial stages. In some cases of MSAP, careful neurological examination may reveal cerebellar ataxia in addition to parkinsonism, suggesting a mixture of underlying parkinsonian and cerebellar pathophysiology. However, the coexistence of parkinsonism in MSAP more often masks the clinical presentation of cerebellar dysfunction, which makes it difficult to detect cerebellar signs associated with MSAP solely on clinical grounds. This issue was previously focused by magnetic cerebellar stimulation (Ugawa et al., 1997), but the stimulation method has not been used widely. Saccades, applicable to a wide range of neurological patients and amenable to quantitative analysis and showing stereotypic manifestations in normal subjects with small variance, can be used to differentiate between these two disorders because the velocity profile of saccades would provide information as to the dysfunction of the cerebellum and basal ganglia in these disorders (Terao et al., 2016, 2017).

From a functional point of view, the superior colliculus (SC) receives projections from both the frontal and parietal cortices. The projection of the frontal cortex to the SC specifies the timing of volitional saccades, either exerting its effect directly or through the basal ganglia, while the projection from the parietal cortex to the SC appears to be more important for determining the saccade metrics (Hikosaka et al., 2000). Saccades are initiated when the SC drives inhibit the activity of the omnipause neurons (OPNs) while releasing that of the medium-lead burst neurons (MLBNs) in the brainstem saccade generator, i.e., excitatory (EBN) and inhibitory burst neurons (IBN), and the eyes are driven approximately toward a target location that corresponds to activity within the SC (Quaia et al., 1999). Saccades terminate when the local OPN activity returns and comes to suppress the burst neurons, while the excitatory signals stop activating the burst neurons (Robinson, 1975; Dean, 1995; Quaia et al., 1999; Buzunov et al., 2013). EBNs and IBNs for horizontal saccades are located in the pontine reticular formation. Thus, the duration of activity in pontine burst neurons strongly correlates with the duration of horizontal saccades (Luschei and Fuchs, 1972).

On the other hand, the cerebellum has been implicated in a role more directly in online control of saccade trajectory (Quaia et al., 1999). The cerebellum is functionally connected with the brainstem oculomotor structures, such as the pontine nuclei, through which it effects its actions; cortical commands arrive at the nucleus reticularis tegmenti pontis (NRTP), and are then sent to the cerebellum, providing spatial information of the target location (Thier and Möck, 2006). The cerebellar output is then projected from the dorsal vermis of the cerebellum back to the pontine burst neurons and OPNs via the cerebellar fastigial nuclei.

The cerebellar output nuclei are considered to adjust the amplitude of the initial pulse of a saccade (Itoh, 2010 for review) by sending signals to accelerate the eye movement up to an appropriate speed and to choke off this speed by an appropriate time, so that the gaze can arrive and stop at the desired location in one step without under/overshoot. More specifically, the cerebellar output acts mainly on the ipsilateral EBNs to accelerate contralateral saccades early during a saccade, and also acts on the contralateral IBNs to decelerate ipsilateral saccades later in the movement, although the two signals exhibit a large temporal overlap (Ohtsuka and Noda, 1991; Fuchs et al., 1993; Robinson et al., 1993; Buzunov et al., 2013). In this way, these neurons modulate the amplitude of saccades.

Cerebellar dysfunction can affect the early accelerating phase, as well as the peak velocity, or the late deceleration phase of saccades (Fuchs et al., 1993; Dean, 1995; Buzunov et al., 2013). Studying velocity profiles of saccades would thus be useful for assessing the accelerating and decelerating (choking) signals of the cerebellum in neurological patients, especially when cerebellar and brainstem pathology is suspected. However, few studies have addressed saccade velocity profiles in neurological disorders. By comparing the velocity profiles, i.e., the peak velocity and accelerating and decelerating phases of MSAP, PD, and normal subjects, we would be able to clarify how the cerebellar and basal ganglia pathways contribute to saccade generation, and how these are affected in neurological disorders.

Pathologically, MSAP affects brainstem structures, including NRTP and the brainstem saccade generator in the reticular formation. Patients with early PD will have a milder pathology in these regions, except for that affecting the substantia nigra and the dorsal vagal nuclei. Thus, we hypothesized that MSAP patients in whom the cerebellar function is impaired would fail to accelerate their saccades to a sufficient level (defective accelerator signal), and they would not be able to decelerate so that the eyes can stop accurately at the target location (defective decelerating signal) (Dean, 1995). Furthermore, they would also show reduced peak velocity due to a defective accelerating signal of the cerebellum as well as to the involvement of the brainstem saccade generator. In contrast, since the cerebellum and its connections to the brainstem would remain functionally intact in PD, they would essentially show normal peak velocity with normal acceleration and deceleration periods (Robinson, 1975; Tada et al., 2015).

We studied a wide range of target eccentricities in order to characterize the velocity profile of saccades more closely than when using a single eccentricity. We also employed two basic oculomotor paradigms: the visually guided saccade (VGS) task, a reflexive saccade, and the memory-guided saccade (MGS) task, a more voluntary saccade. The cerebellum has been postulated to be important in performing visually guided movements such as VGS, whereas cortical as well as basal ganglia commands are considered to play a key role in initiating voluntary movements such as MGS, and to be more prominently affected in basal ganglia disorders including PD (Cerminara et al., 2005; Terao et al., 2011). Thus, by studying these paradigms and using different target eccentricities, we aimed to characterize the signature profiles of saccades to differentiate between PD and MSAP, as both present clinically with parkinsonism but in which cerebellar dysfunction is masked in MSAP.

## 2. Methods

### 2.1. Subjects and clinical assessment

All saccade recordings took place as part of the clinical assessments after we obtained informed consent from the subjects. The experimental procedures were approved by and complied with the guidelines of the local ethics committee. Participants included 63 PD patients (35 males, 28 females, age: 66.6 ± 10.7, range:

42–87) and 16 MSAP patients (11 males, 5 females, age: 61.6 ± 8.2, range: 52–75). We also studied 36 normal subjects (23 males, 13 females, age: 64.2 ± 5.7, range: 55–77) for comparison, who were age-matched with the two patient groups. Subjects whose Mini-Mental State Examination (MMSE) score was below 25 were excluded, as were those who could not follow the task instructions; the latter actually was useful in excluding subjects with cognitive impairments. Table 1 provides the clinical features of the patients studied.

All the MSAP patients were diagnosed and classified as MSAP based on established criteria (Gilman et al., 2008). Furthermore, during the follow-up period of 5 years, most MSAP patients could be distinguished from PD patients on the basis of brain MRI, with features including progressive cerebellar and brainstem atrophy, the "hot cross bun" sign in the pontine basis, and a T2 hyperintense rim at the lateral edge of the dorsolateral putamen.

Since overall, most MSAP patients in this study were at early stages of the disorders, being ambulatory, and presented mainly with parkinsonism at the time of study, while cerebellar ataxia was only minimal in all patients throughout the follow-up period. Their parkinsonian symptoms were difficult to distinguish from those of PD patients in most cases, with no or minimal cerebellar symptoms, whereas autonomic dysfunction appeared in due course. Thus, the disease severity of PD and MSAP patients was evaluated by the Unified Parkinson's Disease Rating Scale (UPDRS) part III or motor score. MSAP and PD patients who participated in this study had comparable UPDRS part III scores ($p > 0.1$, t-test corrected for multiple comparisons by the Bonferroni's method).

Discontinuation of drugs was not feasible for either PD or MSAP patients for ethical reasons. Saccade recording was performed at least 3–4 hours after the drug intake in the morning, according to our previous study, which evaluated saccade performance (VGS and MGS) for several hours after taking L-DOPA (Yugeta et al., 2008); little change was noted in saccade parameters 3 hours after drug intake.

### 2.2. Experimental setup

A PC was used to control the oculomotor paradigms while it stored saccade data for offline analysis (Terao et al., 2018). The subject sat in front of a black, concave dome-shaped screen with a diameter of 90 cm. A chin rest was used to restrain the subjects' heads at a viewing distance of 66 cm. In the dome, light-emitting diodes (LEDs) were embedded in horizontal and vertical arrays, which were used to indicate the locations of the fixation point and targets in the oculomotor paradigms.

According to electo-oculography (EOG) methods previously described (Terao et al., 2016, 2017, 2018), horizontal eye movements were recorded by placing two Ag-AgCl gel electrodes at the lateral angles of both eyes, which was the main focus of this study. Vertical EOG was recorded by placing electrodes over and beneath one eye. Signals input to a DC-amplifier (AN-601G, Nihon-Kohden, Tokyo, Japan) were low-pass filtered at 20 Hz and digitized at 500 Hz. For eye movement calibration, subjects fixated on targets appearing 20 degrees to the left and right of the central spot. We opted to use EOG rather than infrared recording, since the former is very useful in evaluating larger amplitude saccades (20 to 30 degrees) (Mosconi et al., 2010).

### 2.3. Behavioral paradigms

Subjects performed the VGS and MGS tasks. The subject held a microswitch button to begin and end a trial as they pressed or released it. In VGS, a central fixation light was lit immediately after the subjects pressed a button, which they were to foveate. After a period of 1.2–2.0 s, the central fixation light was turned off, and, simultaneously, the target randomly appeared 5, 10, 20, or 30 degrees to the left or right. Subjects were quickly to look at the target. From 0.5 to 1.5 s thereafter, the target point dimmed for 0.5 s. Subjects had to release the button immediately as the dimming occurred. This was to ensure foveation on the target spot. A sound was generated when the button was released within 0.5–1.0 s of the dimming. For failed trials, there was no sound.

In MGS, a trial was also initiated when the subject pressed by the button. While subjects foveated the central fixation point, a peripheral light spot, termed a cue, was lit briefly (50 ms) at the future location of the target, randomly at 5, 10, 20, or 30 degrees to the left or right, and was extinguished shortly after. The subjects were required to remember the location of the target, while they kept fixating the fixation point. At the fixation point offset, the subjects had to make a saccade to the memorized location of the cue. The target point was lit again 600 ms after the offset of the fixation point, and then dimmed. Then, the subjects were instructed to release the button as quickly as possible.

At the beginning of each session, there were five practice VGS and MGS trials. Thereafter, subjects performed 50 test trials of VGS and MGS, in two blocks of 25 trials each.

### 2.4. Data processing

The onset of a saccade was the time when the velocity was greater than 28°/s and acceleration exceeded 90°/s$^2$. After launch, the velocity had to exceed 88°/s for at least 10 ms. The end of a saccade was the time when the velocity fell below 40°/s. The duration of a saccade had to exceed 30 ms. Trials with noise in the records and those with a latency under 100 ms were discarded. MGS results with a latency longer than 660 ms were also excluded from statistical analyses (see below; Terao et al., 2016, 2017).

In this study, we aimed to characterize the velocity profiles of VGS and MGS in PD and MSAP patients in comparison to normal subjects. Only the first saccade after the offset of the fixation point was analyzed. For each trial, parameters of the velocity profile were peak velocity and duration. The duration of a saccade was further divided in its acceleration and deceleration periods, i.e., measured from the time of saccade onset to peak velocity and from the time of peak velocity to the end of the saccade.

In order to compare the saccade velocity profiles among different subject groups, the mean and median of peak velocity and acceleration and deceleration periods in VGS and MGS were calculated across and separately for different target eccentricities (Table 2). Statistical analyses were conducted using a commercial software package, SPSS statistics 17.0.0 (SPSS Japan, Inc., Tokyo). Repeated-measures analysis of variance (rmANOVA) was

**Table 1**
Patient characteristics.

| Subject group | No. of cases | Male | Female | Age (yrs) | Disease duration (yrs) | UPDRS motor score |
|---|---|---|---|---|---|---|
| Normal | 36 | 23 | 13 | 64.2 ± 5.7 | – | – |
| PD | 63 | 35 | 28 | 66.6 ± 10.7 | 6.2 ± 5.2 | 26.4 ± 11.3 |
| MSAP | 16 | 11 | 5 | 61.6 ± 8.2 | 3.3 ± 1.5 | 31.5 ± 12.5 |

**Table 2**
Correlation between the parameters of saccade velocity profile and disease severity.

| | VGS | | | | MGS | | | |
|---|---|---|---|---|---|---|---|---|
| **Peak velocity** | **5°** | **10°** | **20°** | **30°** | **5°** | **10°** | **20°** | **30°** |
| PD | r = 0.014, p = 0.9291 | r = 0.080, p = 0.6135 | r = −0.002, p = 0.9895 | r = 0.057, p = 0.7166 | r = 0.018, p = 0.9294 | r = 0.011, p = 0.9564 | r = 0.049, p = 0.8067 | r = −0.014, p = 0.9442 |
| MSA-P | r = 0.274, p = 0.4910 | r = 0.236, p = 0.5551 | r = 0.220, p = 0.5841 | r = 0.201, p = 0.6177 | r = −0.531, p = 0.0004 | r = −0.359, p < 0.0001*** | r = −0.301, p < 0.0001*** | r = −0.564, p < 0.0001*** |
| **Acceleration phase** | **5°** | **10°** | **20°** | **30°** | **5°** | **10°** | **20°** | **30°** |
| PD | r = 0.096, p = 0.5408 | r = −0.021, p = 0.8935 | r = −0.185, p = 0.2357 | r = 0.184, p = 0.2383 | r = 0.281, p = 0.1488 | r = 0.082, p = 0.6814 | r = 0.113, p = 0.5702 | r = −0.068, p = 0.7325 |
| MSA-P | r = 0.584, p = 0.1016 | r = −0.315, p = 0.4251 | r = −0.353, p = 0.3660 | r = −0.156, p = 0.6998 | r = 0.180, p = 0.1489 | r = 0.469, p < 0.0001*** | r = 0.300, p = 0.0141* | r = 0.210, p = 0.0911 |
| **Deceleration phase** | **5°** | **10°** | **20°** | **30°** | **5°** | **10°** | **20°** | **30°** |
| PD | r = 0.161 p = 0.3032 | r = 0.360 p = 0.0172* | r = 0.109, p = 0.4895 | r = 0.058, p = 0.7147 | r = 0.257, p = 0.0476* | r = 0.369, p = 0.0034** | r = 0.417, p = 0.0008** | r = 0.503, p < 0.0001*** |
| MSA-P | r = −0.145, p = 0.7208 | r = −0.062, p = 0.8796 | r = 0.130, p = 0.7479 | r = −0.119, p = 0.7689 | r = −0.046, p = 0.7833 | r = 0.699, p < 0.0001*** | r = 0.593 p < 0.0001*** | r = 0.464, p < 0.0001*** |

Left half: VGS, right half: MGS. Asterisks (*) depict significant correlations.
* :p < 0.05.
** :p < 0.01.
*** :p < 0.0001.

performed on the parameters of saccade velocity profiles, with subject group (three levels: normal subjects, PD, and MSAP patients) as a between-subject factor, and target location (four levels: 5, 10, 20, and 30 degrees) as a within-subject factor. Bonferroni's correction was used for multiple comparisons. We correlated disease severity assessed by UPDRS part III with the saccade parameters for each patient group using the Spearman's rank-order correlation. The significance criterion was set at $p < 0.05$.

Saccades in patients with parkinsonism and cerebellar ataxia frequently show reduced amplitude (hypometria), whereas some patients are known to show slowed saccade velocity (see Terao et al., 2016, 2017 for review). Because the peak velocity and duration of saccades depend on their amplitude, saccades of similar sizes were collected in order to compare the velocity profiles of saccades among different subject groups (Bahill et al., 1975). Namely, for 5-degree targets, trials with a saccade size between 2.5 and 7.5 degrees were pooled. Similarly for 10-, 20-, and 30-degree targets, trials with saccade sizes between 7.5–12.5 degrees, 17.5–22.5 degrees, and 27.5–32.5 degrees were pooled. As a result, the amplitudes of saccades collected for each target category were comparable among the three groups at all target eccentricities ($p > 0.05$).

In order to evaluate the reduction of saccade velocity with respect to saccade amplitude, we also analyzed the relationship between saccade amplitude and the peak velocity (main sequence; Bahill et al., 1975), expressed by the equation below:

$$V_{max} = V_0 X[1 - \exp(-A_s/A_0)]$$

where $V_{max}$ gives the maximal saccade velocity (peak velocity) and $A_s$ gives the saccade amplitude. We determined $V_0$ and $A_0$ by curve fitting (see below). According to Bahill et al. (1975), saccade trials for VGS and MGS were pooled in each subject, and we constructed a scattergram plotting the peak velocity against the saccade amplitude (Terao et al., 2016, 2017). Thereafter, fitting to the above equation was done using the logistic curve fitting function of a commercial software program (OriginPro 2018, Lightstone, Tokyo, Japan). Goodness of fit was given by $R^2$ adjusted for the degree of freedom. Once it is successfully fitted with the scattergram, the curve courses through the origin. The asymptotic value corresponds to $V_0$, while $V_0/A_0$ would represent the slope of the tangent line at the origin. Repeated measures ANOVA was performed to study if the main sequence differs among groups with respect to $V_0$ and $V_0/A_0$, again with subject group and target eccentricity as between-subject and within-subject factors. Based on these data ($V_0$ and $V_0/A_0$), the receiver operating curve (ROC) analysis was also performed to see whether the asymptotic velocity ($V_0$) and main sequence slope ($V_0/A_0$) could distinguish MSA-P from PD again by the same software.

## 3. Results

As shown in our previous studies, both MSAP and PD patients showed hypometria in both VGS and MGS (Fig. 1, left traces). The right traces in Fig. 1A give the velocity profile of saccades, corresponding to the saccade traces on the left side (Fig. 1A: VGS, Fig. 1B: MGS). Fig. 1C shows the plot of the grand average trace, with a separate line for each patient group and shaded areas depicting one standard error across subjects. Compared to normal subjects, PD patients showed a smaller peak velocity. The acceleration period (time from start to peak of a saccade) was normal, whereas the deceleration period was prolonged in the PD patient, especially for MGS of larger amplitudes at 20 and 30 degrees. MSAP patients showed a much more reduced peak velocity compared to normal controls, with longer acceleration and deceleration periods.

Fig. 2 shows the frequency distribution of saccades across trials, in which the amplitude of saccades in individual trials is plotted against the latency (left, VGS; right, MGS). The plot depicts the frequency with which the combination of latency and amplitude is seen among the pooled saccade data across all subjects, which was expressed as a contour map, with the most frequent combinations shown in the darkest color. Again, saccades of MSAP and PD patients were hypometric relative to normal subjects for all target eccentricities, with few trials showing hypermetria (compared with Fig. 2 in Terao et al., 2017). Both saccade latency and amplitude were also more variable in the patient groups than in normal subjects, especially for MSAP patients.

Figs. 3 and 4 plot the acceleration and deceleration periods against the saccade amplitude across individual trials for VGS and MGS, respectively. Each dot in the plots corresponds to one trial. MSAP patients were shifted towards longer acceleration period (upper plots) compared to normal subjects and PD patients, especially for larger saccade amplitudes (20–30 degrees), whereas the latter two groups showed similar distributions. In contrast, the deceleration period of MSAP patients (lower plots) was more variable and prolonged than that of normal subjects across target eccentricities. PD patients also showed slightly longer deceleration periods than normal subjects did, but only at 20–30 degrees. To confirm the observations obtained by visual inspection of these plots, we perform statistical analyses on the parameters of saccade velocity profile in the next section.

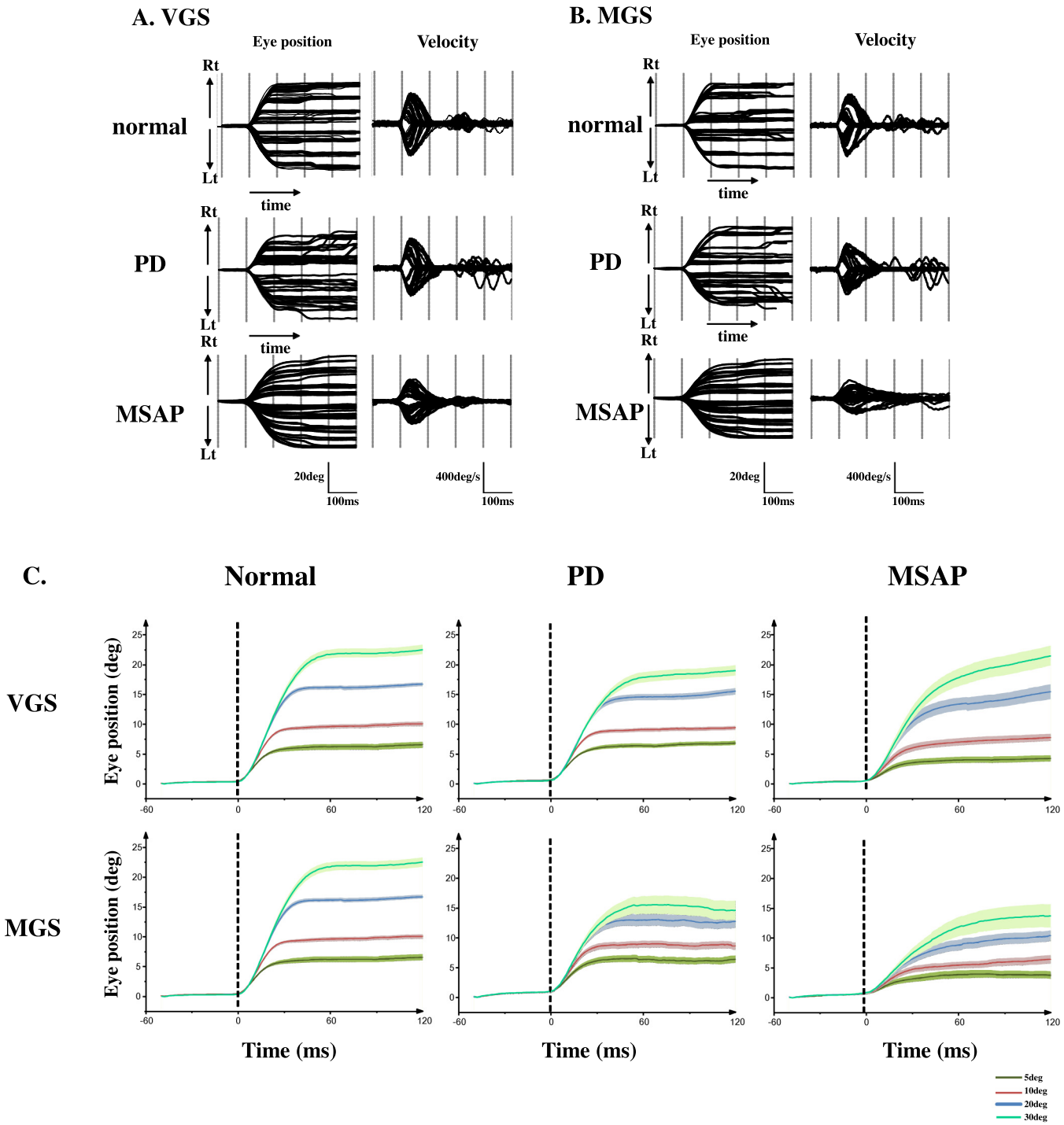


**Fig. 1. Saccade traces in normal subject and PD and MSAP patients.** VGS (A) and MGS (B) traces in a normal subject (top), a PD patient (middle), and an MSAP patient (bottom). Traces of 20–30 trials of saccades are overlapped and aligned at the saccade onset. Left column: eye position, right column: saccade velocity profile. The ordinate shows the eye position and abscissa gives the time axis. Interval between vertical lines: 100 ms. C. Grand average mean trace of each patient group. Shaded areas indicate one standard error across subjects above and below the mean. The ordinate shows the eye position and abscissa gives the time axis. Interval between vertical lines: 100 ms. Dashed vertical lines indicate onset of saccades. Note that for PD and MSAP patients, the amplitude of saccades is smaller in MGS as compared to VGS.

To summarize, both PD and MSAP patients showed hypometria both in VGS and MGS, with more variable latency and amplitude than in normal subjects, especially for MSAP. MSAP patients showed a reduced peak velocity, with both increased acceleration and deceleration periods. In contrast, in PD patients, the peak velocity and the acceleration period were normal, whereas the deceleration phase was prolonged.

### *3.1. VGS and MGS velocity profile in PD and MSAP*

*VGS*

We compared the saccade velocity profiles among subject groups, especially with respect to different target eccentricities (Fig. 5, Supplementary Table 1). As described in the methods, in order to compare the velocity profile of saccades among different

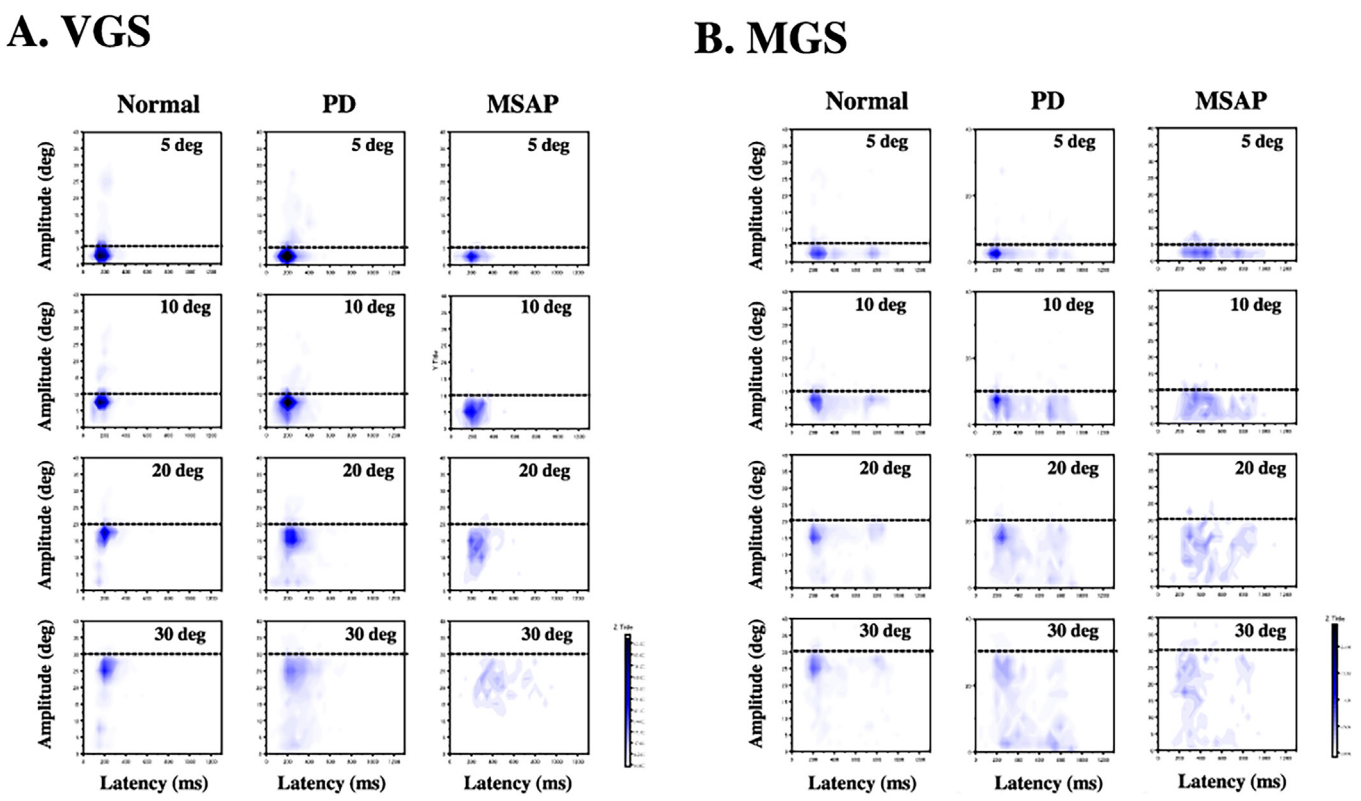


**Fig. 2. Frequency distribution of saccade latency and amplitude.** The figure visualizing the frequency distribution of saccade latency and amplitude pooled across total trials in all subjects: normal subjects, PD, and MSAP patients. x-axis: latency (ms), y-axis: amplitude (deg). This was displayed as contour maps, in which the darkest regions indicate the most frequently encountered combinations of saccade latency and amplitude and the lighter regions indicate less frequent combinations. (For gradation of the contour map, see the panel linked to the figure.) Horizontal dashed lines in each plot depict the target eccentricity. Fig. 2A is for VGS and 2B for MGS.

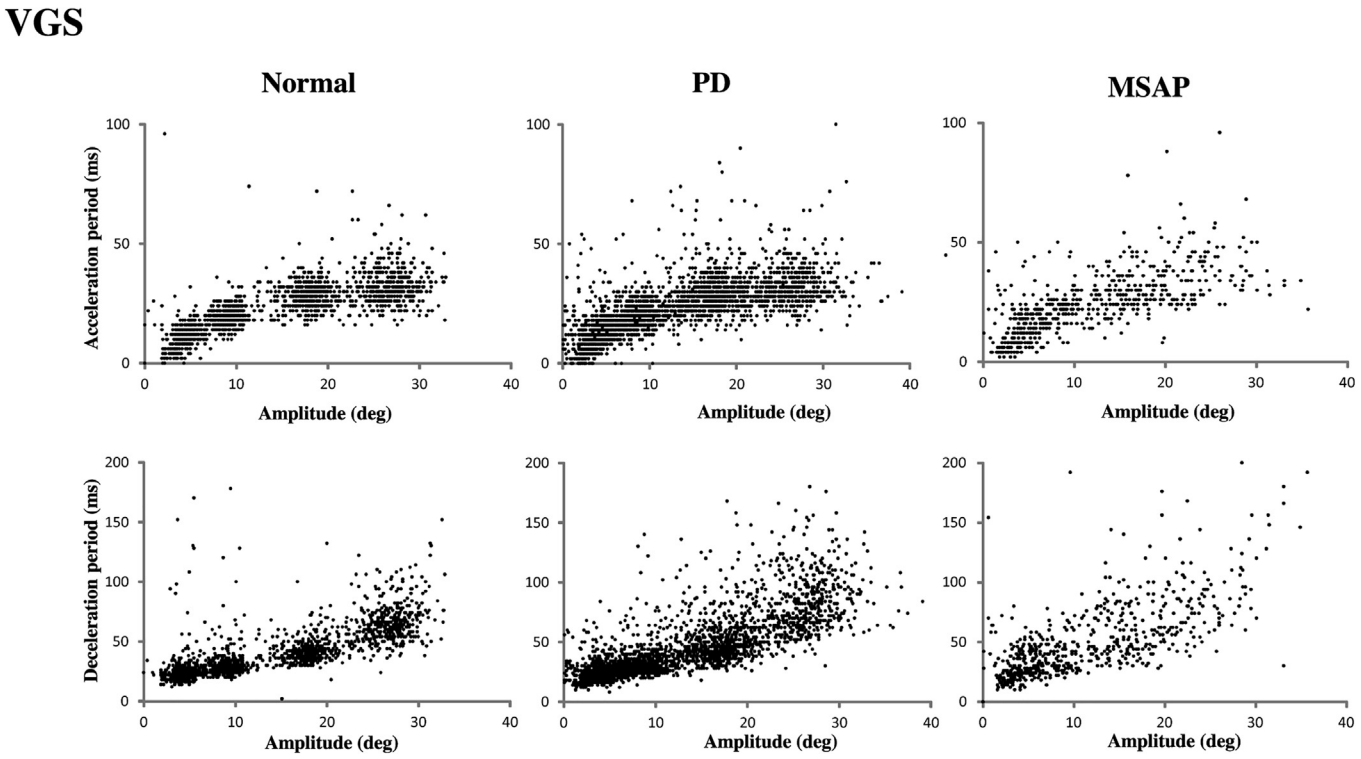


**Fig. 3. Scatterplot of acceleration and deceleration periods versus saccade amplitude for VGS.** Data for the acceleration and deceleration periods (ms) of VGS in individual trials were pooled across subjects and plotted against the saccade amplitude. Each dot in the plots represents a single trial.

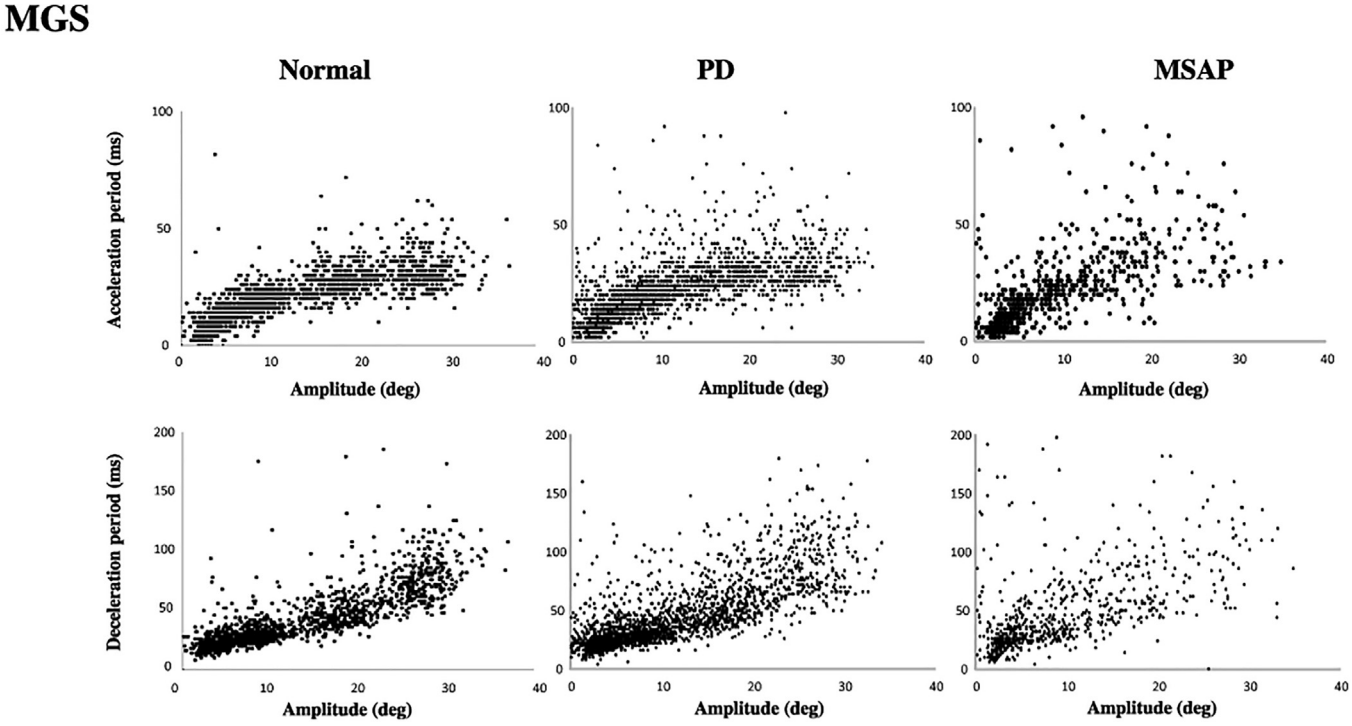


**Fig. 4. Scatterplot of acceleration and deceleration versus saccade amplitude for MGS.** A similar scatterplot (as in Fig. 3) generated for MGS.

groups, we selected only those saccades whose amplitudes were comparable for each target eccentricity (see Methods).

Repeated measures ANOVA (rmANOVA) performed on the peak velocity showed that both the effect of group and that of target eccentricity was significant, without significant interaction between them (effect of target group: $F[2,224] = 11.583$, $p < 0.0001$; effect of target eccentricity: $F[3,336] = 305.355$, $p < 0.0001$; group X eccentricity: $F[6,672] = 1.435$, $p = 0.2020$). This indicated that MSAP patients showed a slower peak velocity of VGS than did normal subjects and PD patients (Fig. 5A, upper figures). The peak velocity increased with increasing target eccentricities, similarly for all groups.

rmANOVA performed on the acceleration period indicated significant main effects of target eccentricity and of group, with no significant interaction between them (effect of target eccentricity: $F[3,336] = 327.448$, $p < 0.001$; effect of group: $F[2,224] = 17.606$, $p < 0.0001$; group X eccentricity: $F[6,672] = 1.553$, $p = 0.1616$). This suggested that MSAP patients had a longer acceleration period, overall, compared to normal subjects and PD patients, while the acceleration period was comparable between normal subjects

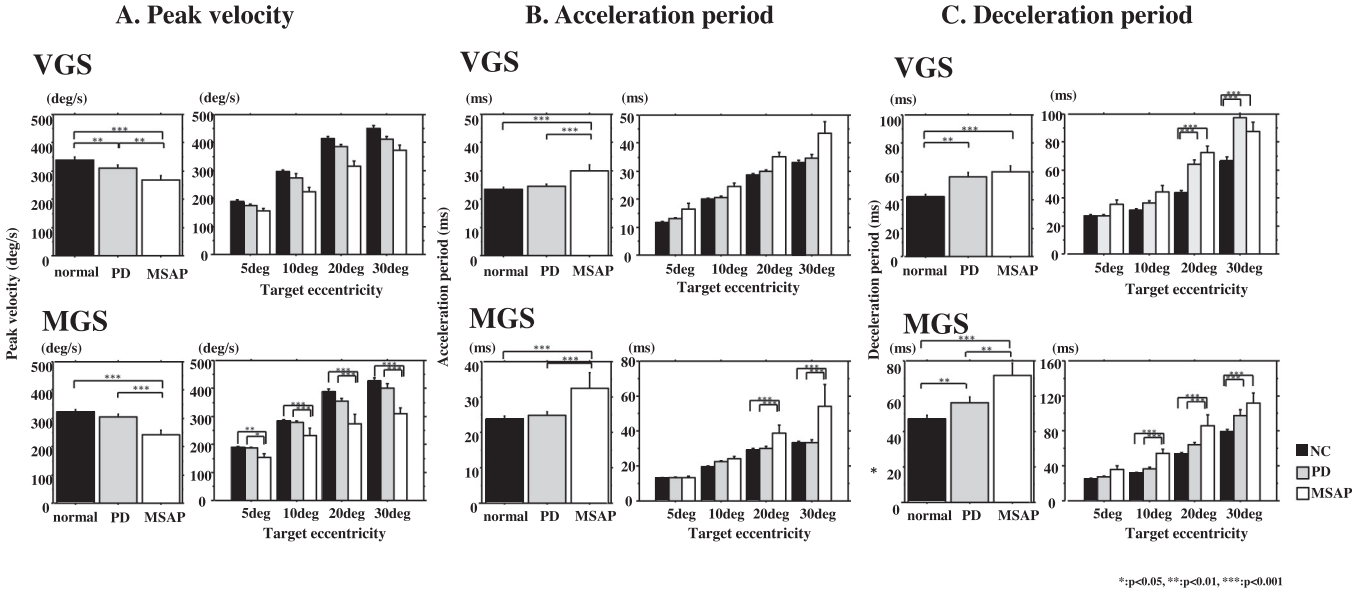


**Fig. 5. Parameters of saccade velocity profiles at different target eccentricities.** Bar graphs depicting the peak velocity (A), acceleration (B), and deceleration periods (C) of saccades for each target eccentricity in the three subject groups. Upper figures: VGS, lower figures: MGS. Black bars: normal subjects, gray bars: PD patients, white bars: MSAP patients. Figures on the left side show parameters averaged across all target eccentricities. Figures on the right side show parameters calculated separately for different target eccentricities. Error bars indicate standard errors. *:$p < 0.05$, **:$p < 0.01$, ***:$p < 0.001$.

and PD patients at all target eccentricities (t-test corrected for multiple comparisons by the Bonferroni's method: $p > 0.05$). The acceleration period of VGS increased with increasing target eccentricity, similarly for all subject groups (Fig. 5B, upper figures).

As regards the deceleration period, rmANOVA showed significant a main effect of target eccentricity as well as that of group, with a significant interaction between them (effect of target eccentricity: $F[3,336] = 191.176$, $p < 0.0001$; effect of group: $F[2,224] = 21.811$, $p < 0.0001$; group X eccentricity: $F[6,672] = 10.597$, $p < 0.0001$). This implied that the deceleration period of VGS in both MSAP and PD patients was longer compared to normal subjects, whereas the two patient groups were similar on average (Fig. 5C, upper figures). The deceleration period of MSAP and PD patients increased with the target eccentricity, and deviated more from that of normal subjects at larger target eccentricities (20–30 degrees).

*MGS*

rmANOVA performed on the peak velocity of MGS showed significant main effects of target eccentricity and of group, with a significant interaction between the two factors (effect of target eccentricity: $F[3,336] = 305.355$, $p < 0.0001$; effect of group: $F[2,224] = 11.250$, $p < 0.0001$; effect of group: $F[2,224] = 11.250$, $p < 0.0001$; group X eccentricity: $F[6,672] = 5.009$, $p < 0.0001$). This reflected the fact that MSAP patients showed a slower peak velocity of MGS than normal subjects and PD patients did (Fig. 5A, lower figures). The peak velocity of MGS increased with increasing target eccentricities for all groups, but more so for normal subjects and PD patients.

As regards the acceleration period of MGS, there was a significant main effect of target eccentricity as well as that of group, with no significant interaction between them (Fig. 5B, lower figures; effect of target eccentricity: $F[3,336] = 131.686$, $p < 0.001$; effect of group: $F[2,224] = 17.370$, $p < 0.0001$; group X eccentricity: $F[6,672] = 5.856$, $p = 0.0020$). Namely, the acceleration period of MGS was overall more prolonged in MSAP than in PD patients and normal subjects, whereas it was nearly comparable for PD patients and normal subjects at all target eccentricities. In contrast, the difference between MSAP patients and the other two groups grew with increasing target eccentricity, becoming significant at 20–30 degree target eccentricity (post-hoc analysis: MSAP vs. normal: $p < 0.0001$, MSAP vs. PD: $p < 0.0001$).

rmANOVA conducted on the deceleration period of MGS showed a main effect of target eccentricity as well as that of group (Fig. 5C, lower figures; effect of target eccentricity: $F[3,336] = 227.784$, $p < 0.001$ effect of group: $F[2,224] = 14.524$, $p < 0.0001$; group X eccentricity: $F[6,672] = 3.614$, $p = 0.0020$). This implicated that the deceleration period of MGS was longest in MSAP, intermediate in PD patients, and smallest in normal controls, and the difference between MSAP and PD patients as well as that between PD patients and normal subjects was significant. It increased with increasing target eccentricity for all subject groups. The deceleration period of PD patients, being comparable to normal subjects at 5 degrees, gradually caught up with that of MSAP patients at 30 degrees.

To summarize, the acceleration periods of PD for both VGS and MGS were comparable to that of normal subjects, whereas the deceleration period were mildly prolonged compared with normal subjects. MSAP patients showed both prolonged deceleration and acceleration periods for both MGS and VGS compared with normal subjects. In addition, MSAP patients showed a decreased peak velocity compared with normal subjects and PD patients.

### 3.2. Correlation between parameters of velocity profile and severity of parkinsonism

We correlated the parameters of the saccade velocity profile, i.e., the peak velocity, as well as acceleration and deceleration periods, with the severity of parkinsonism as assessed by the UPDRS motor score (Table 2).

*VGS*

In PD patients, the peak velocity of VGS did not show any significant correlation with the disease severity (assessed by the UPDRS motor score) at any target eccentricity (Fig. 6A, upper figure; Table 2). Correlation was not significant between the disease stage and the acceleration period of VGS (Fig. 6B, upper figure). Neither did the disease stage correlate significantly with the deceleration period at any target eccentricities (Fig. 6C, upper figure).

Similarly, in MSAP patients, although the peak velocity of VGS appeared to decline with advancing disease, it did not correlate significantly with the disease severity at any target eccentricity (Fig. 6A, lower figure; Table 2). Meanwhile, although the acceleration and deceleration periods of VGS appeared to increase slightly with advancing disease, there was no significant correlation between the acceleration period of VGS and disease severity (Fig. 6B, lower figure), nor was there any correlation between the deceleration period of VGS and disease severity at any of the target eccentricities (Fig. 6C, lower figure).

*MGS*

In PD patients (Fig. 7, upper figures), the peak velocity of MGS did not show any significant correlation with the disease severity (assessed by the UPDRS motor score), although it appeared to show a trend to increase slightly with disease severity at 30-degree target eccentricity (Fig. 7A, upper figure). Correlation was not significant between the disease severity and the acceleration period of MGS at any eccentricities (Fig. 7B, upper figure). However, the disease severity correlated positively with the deceleration period at all target eccentricities, especially at target eccentricities of 20 and 30 degrees (Fig. 7C, upper figure; Table 2).

In MSAP patients (Fig. 7, lower figures), the peak velocity of MGS showed a relatively weak but moderate to strong significant negative correlation with the disease stage at all eccentricities (Fig. 7A, lower figure). Also, the acceleration period of MGS and disease severity (as assessed by the UPDRS motor score) correlated positively at 10 and 20 degrees and exhibited a trend for correlation at 30 degrees (Fig. 7B, lower figure). Similarly, the deceleration period of MGS correlated moderately to strongly with disease severity at 10-, 20-, and 30-degree eccentricities, but not at 5 degrees (Fig. 7C, lower figure).

In summary, parameters characterizing the velocity profile of VGS, i.e., the peak velocity as well as the acceleration and deceleration periods of VGS, did not correlate significantly with the disease severity (parkinsonism) in either MSAP or PD patients. In contrast, the longer acceleration and deceleration periods and a slower peak velocity in the velocity profile of MGS in MSAP patients correlated with the severity of parkinsonism at most target eccentricities, whereas the velocity profile of PD patients was characterized by a slightly longer decelerating period, which also correlated mildly with disease severity.

### 3.3. The main sequence relationship in PD and MSAP patients

We plotted the peak velocity of VGS against saccade amplitude for each trial in each subject, which generally showed a good fit with the main sequence relationship (Fig. 8; adjusted $R^2$: normal, $0.858 \pm 0.025$; PD, $0.795 \pm 0.019$; MSAP, $0.858 \pm 0.025$). Comparing the main sequence relationships with a fair fit of above 0.8, both the asymptoted velocity ($V_0$) of the fitting curves tended to be smaller in MSAP than in normal subjects and PD patients, whereas it was comparable between the latter two groups ($V_0$: effect of group: $F[2,224] = 4.736$, $p = 0.0106$; normal: $447.2 \pm 17.2$ deg/s; PD: $399.0 \pm 9.7$ deg/s; MSAP: $380.0 \pm 21.4$ deg/s). The slope at origin ($V_0/A_0$) was also smaller in MSAP patients than in normal subjects and PD patients ($p < 0.0006$), whereas those of normal

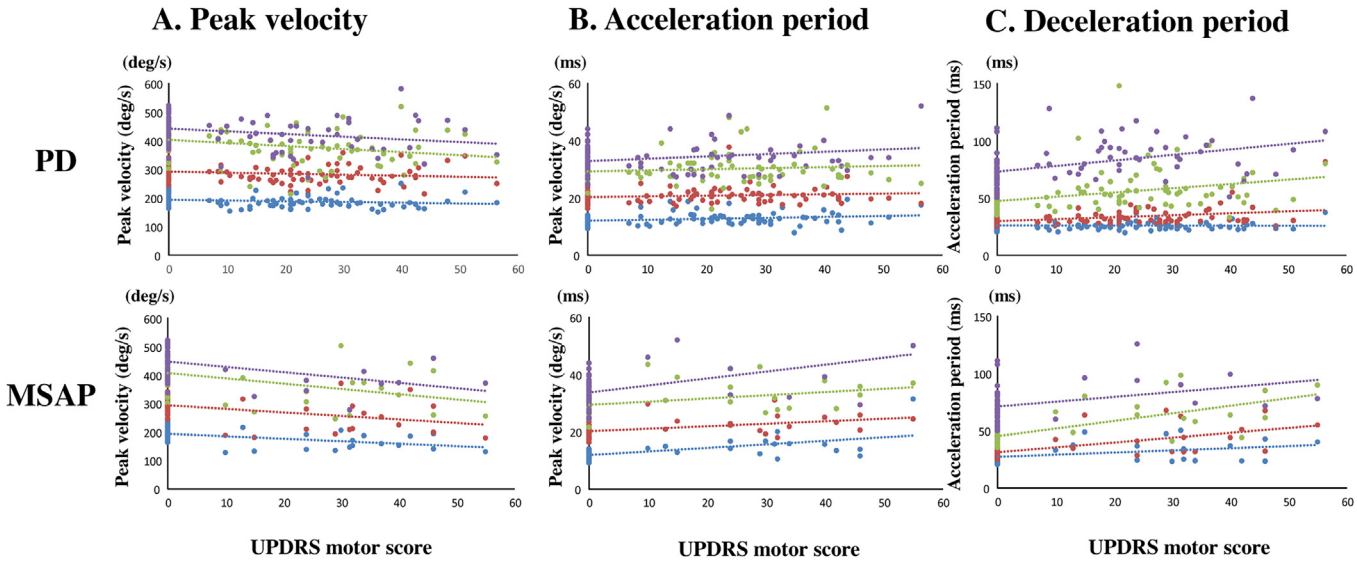


**Fig. 6. Correlation of parameters of saccade velocity profiles of VGS with disease severity.** Scatterplots in which the parameters of saccade velocity profile (ordinate) were plotted separately for different target eccentricities against the severity of parkinsonism as assessed by the UPDRS motor score (abscissa). A: Peak velocity, B: Acceleration period, C: Deceleration period. Each dot in the plots represents data of each subject. Blue dots: 5 deg, red dots: 10 deg, green dots: 20 deg, purple dots: 30 deg.

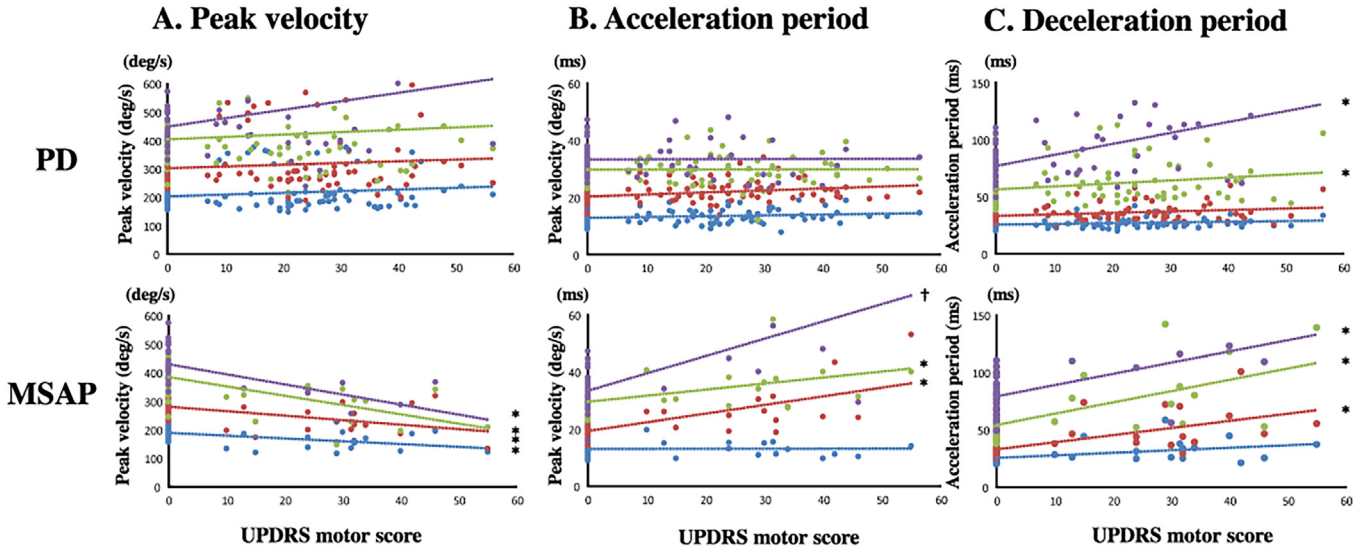


**Fig. 7. Correlation of parameters of saccade velocity profiles of MGS with disease severity.** Similar scatterplots for MGS. Conventions as in Fig. 6. Significant correlations with disease severity are indicated by asterisks (*). † indicates a trend for correlation.

subjects and PD patients were statistically comparable ($V_0/A_0$: effect of group $F[2,224] = 12.043$, $p < 0.0001$; normal: 52.3 ± 1.7/s, PD: 57.1 ± 1.2/s, MSAP: 43.9 ± 2.8/s).

The scatterplot of peak velocity of MGS versus the saccade amplitude also showed a fair fit with the main sequence equation in each subject (adjusted $R^2$: normal, 0.861 ± 0.016; PD, 0.734 ± 0.024; MSAP, 0.696 ± 0.037). Comparing the main sequence relationships with a fair fit of above 0.7, MSAP patients showed significantly smaller $V_0$ than normal subjects and PD patients ($V_0$: effect of group: $F[2,224] = 14.390$, $p < 0.0001$; normal: 418.8 ± 11.8 deg/s, PD: 382.5 ± 9.8 deg/s, MSAP: 296.6 ± 19.5 deg/s), as was $V_0/A_0$ (effect of group $F[2,224] = 6.630$, $p = 0.0019$; normal: 54.5 ± 1.2/s, PD: 57.0 ± 1.2/s, MSAP: 47.6 ± 3.2/s), whereas $V_0$ and $V_0/A_0$ were statistically comparable for normal subjects and PD patients. Thus, MSAP patients showed significantly reduced peak velocity when considering saccade amplitude, but PD patients showed a normal velocity-amplitude relationship.

Based on the data obtained from curve fitting, $V_0$ and $V_0/A_0$, the receiver operating curve (ROC) analysis was performed to see whether these parameters could distinguish MSA-P from PD. For VGS, the asymptote value of velocity ($V_0$) and main sequence slope ($V_0/A_0$) showed an area under curve (AUC) of 0.580 ($p = 0.0792$) for $V_0$ and 0.797 ($p < 0.0001$) for $V_0/A_0$, respectively, suggesting a moderate discriminative power to distinguish MSA-P from PD patients for $V_0/A_0$ but not $V_0$. For MGS, both $V_0$ and main sequence slope $V_0/A_0$ showed a moderate power to distinguish MSA-P from PD patients. (V0: AUC 0.770, $p < 0.0001$, V0/A0: AUC 0.753, $p < 0.0001$).

## 4. Discussion

Saccades of both PD and MSAP patients showed hypometria, consistent with parkinsonism, which was more pronounced in

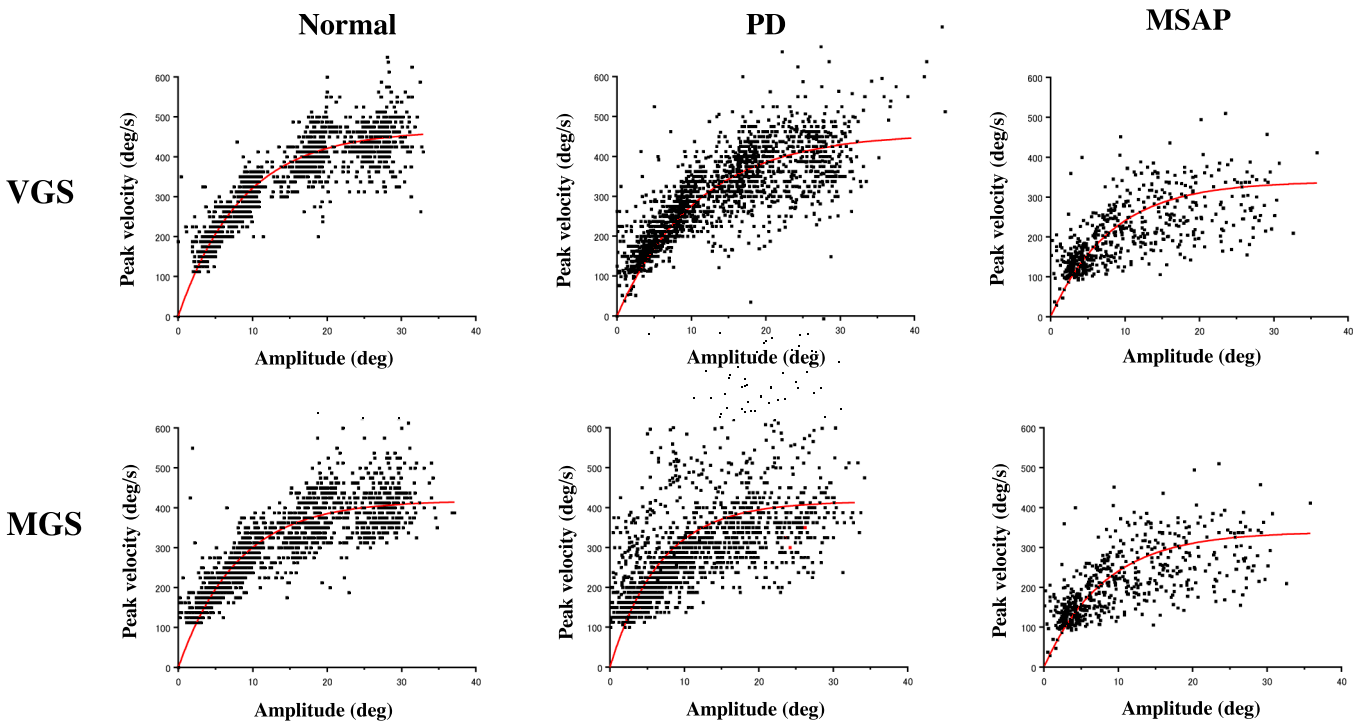


**Fig. 8. Relationship between saccade amplitude and peak velocity in each subject group.** The figure shows a scatterplot of peak velocity versus the saccade amplitude for the data across subjects, pooled separately in each group (normal subjects, PD and MSAP patients). Each dot represents one trial. Curve fitting using the equation depicted in the methods are overlaid on the plots. Note that actual curve fitting was done for data for individual subjects, separately, instead of on the pooled data across subjects in each group.

MSAP. As noted in our previous study (Terao et al., 2016), hypometria correlated with disease severity in both patient groups. Although hypermetria was frequently observed in SCD patients (Terao et al., 2017), it was almost never seen in our MSAP patients (Figs. 1 and 2), despite underlying olivopontocerebellar pathology. However, MSAP patients exhibited more variability in latency and amplitude as well as in the acceleration and deceleration periods than normal subjects and PD patients (Fig. 2), possibly reflecting combination of cerebellar dysfunction with parkinsonism in these patients.

Despite similar clinical presentations of PD and MSAP, the velocity profiles differed largely in the two disorders. MSAP patients showed reduced peak velocity, even when considering the amplitude, with increased acceleration and deceleration periods. In contrast, PD patients showed normal acceleration and peak velocity, but a prolonged deceleration period. The difference between the two groups became more prominent with increasing target eccentricity.

Finally, for MGS but not VGS, parameters such as peak velocity and acceleration and deceleration periods correlated significantly with the disease severity of parkinsonism assessed by the UPDRS motor score in both PD and MSAP patients.

### *4.1. Pathophysiology underlying the abnormal velocity profiles of MSAP*

As mentioned in the Introduction, the impaired accelerating signal would impact the early accelerating phase of the saccade velocity profile and also the peak velocity, while the impaired decelerating signal of saccades would influence the late deceleration phase of a saccade. It has been suggested that both these signals come from the cerebellar output nuclei (Buzunov et al., 2013; Quaia et al., 1999).

The velocity profile of MSAP patients was also characterized by a slow peak velocity, even when considering its amplitude, in addition to prolonged acceleration and deceleration periods (Fig. 8), which deteriorated with disease severity (parkinsonism). The reduced peak velocity and prolonged acceleration periods together would point to a diminished acceleration signal.

According to Quaia et al. (1999), the accelerating signal corresponds to the sum of the fastigial oculomotor regions and the collicular inputs to excitatory burst neurons (MLBNs). Here, the collicular acceleration drive, which determines the initial saccade direction, is supposed to be stronger than that from the cerebellar pathway. MSA is characterized by brainstem pathology, such as the pontine nuclei and reticular formation, whereas the cerebellar cortex and its output nuclei are comparatively preserved (Wakabayashi et al., 2005). This should lead to the diminished accelerating drive brought about by impaired saccade generator function, in which it takes longer to accelerate even up to a reduced level of peak velocity (i.e., longer acceleration period). Thus, the reduced peak velocity of MSAP patients would be ascribed not only to cerebellar pathology leading to impaired drive arriving from the cerebellar output nuclei, but also to brainstem pathology, which diminishes the directional drive; the diminished peak velocity was also seen in MSAC patients, which is also characterized by brainstem as well as cerebellopetal pathology. Pathophysiologically, in parkinsonism (both PD and MSAP), the overactive output nuclei of basal ganglia, the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr), inhibit downstream structures of the motor and oculomotor systems (Hikosaka et al., 2000). This suppression of SC would further diminish the accelerating drive arriving at the brainstem saccade generator from the SC (Figs. 3–5, Machado and Rafal, 2004). Due to these compound causes for the reduction of accelerating signal, the peak velocity of MSAP patients was even more slowed than in MSAC patients (see Terao et al., 2017).

MSAP patients also showed longer deceleration periods as compared to normal subjects. In our previous study (Terao et al., 2017), we compared saccade velocity profiles between MSAC and SCD patients and found a prolonged deceleration period in SCD but not MSAC patients. The prolonged deceleration period was ascribed to the cerebellar dysfunction, more specifically, defective decelerating signal from the cerebellar output (cerebellofugal) pathways in SCD (Gitchel et al., 2013; Hanajima et al., 2016). However, MSAC patients who share a cerebellopetal pathology and show a more prominent cerebellar symptom than MSAP patients, actually exhibit a shortened instead of prolonged acceleration period and normal deceleration period (Terao et al., 2017; see next section). Thus, cerebellar dysfunction may lead to different patterns of acceleration and deceleration periods according to potentially different cerebellar involvement noted in SCD, MSAC and MSAP. Alternatively, the prolonged acceleration period may be explained by the impaired saccade generator function as discussed previously, but again, this period was shortened in MSAC; due to the largely diminished accelerating drive, more time would be required to reach a low level of peak velocity, hence a longer acceleration period. Since reduction of acceleration drive was more pronounced for MSAP than MSAC, the acceleration periods in MSAP was longer.

### 4.2. Comparison of velocity profiles between MSAC and MSAP

To look further into the pathophysiology underlying the saccade velocity profiles, we compared the velocity profiles of MSAP with those of MSAC patients who were investigated in our previous study (Terao et al., 2017). MSAC and MSAP compose a common pathological continuum, both representing synucleinopathy with substantial overlap in pathological features, differing only in its distribution (Tada et al., 2015). The difference in distribution, the striatonigral system for MSAP and olivopontocerebellar system for MSAC (Ozawa et al., 2010; Tada et al., 2015), is considered to explain the different clinical presentations and the difference in saccade profile.

As noted above, the acceleration period was prolonged in MSAP, but was shortened in MSAC (see Terao et al., 2017). According to the clinicopathological contrast between MSAC and MSAP, the prolonged acceleration as found in MSAP may be associated with parkinsonism or pathology within the striatonigral system. Although it should be acknowledged that any clinical correlation may simply reflect advancing disease, significant positive correlation was noted between the deceleration phase and the disease stage or between the acceleration period and the disease stage in MSAP (but only for MGS), whereas in MSAC, no correlation was noted between the acceleration/deceleration phases and the disease stage (Terao et al., 2017).

The deceleration period was also prolonged in MSAP, while it was comparable to that of normal subjects in MSAC patients (Terao et al., 2017). Furthermore, the deceleration period correlated significantly with advancing disease severity, but only for MGS in MSAP patients at 10, 20, and 30 degrees. In contrast, no correlation was noted between the acceleration/deceleration phases and the disease stage in MSAC (Terao et al., 2017). Again, according to the contrast between MSAC and MSAP, the prolonged acceleration and deceleration periods in MSAP, especially for MGS at larger target eccentricities, may be related to pathology within the striatonigral system, present in MSAP.

Recent experiments in monkeys suggest that SNr modulates SC activity by acting on the inhibitory circuit within the SC, causing hypometric saccades (Liu and Basso, 2008). If the burst neurons of SC are experimentally inhibited by local injection of muscimol (Hikosaka and Wurtz, 1985a), by bicuculline injection into its intermediate layer (Hikosaka and Wurtz, 1985b), or by electrical stimulation of the SNr (Liu and Basso, 2008), the discharge rates of SC burst as well as those of buildup neurons are reduced. Since the bursts of SC neurons correlate significantly with the peak saccade velocity, reduced firing rates of SC neurons would result in decreased amplitude of saccades and reduced peak velocity with increased duration in addition to delayed or even suppressed saccade initiation (van Gisbergen et al., 1981; Hikosaka and Wurtz,1985a,b; Aizawa and Wurtz, 1998). The effects of SC inhibition by muscimol injection or by SNr stimulation are much larger on MGS than on VGS (Hikosaka and Wurtz, 1985a,b; Liu and Basso, 2008), explaining the greater changes in velocity profile for MGS as compared to VGS in the present study (Fig. 1).

### 4.3. Pathophysiology underlying the abnormal velocity profiles of PD

In contrast to MSAP, the accelerating signal in PD patients was normal, as indicated by the acceleration time and peak velocity statistically similar to normal subjects (Figs. 1, 3–5), and the preserved main sequence relationship, whereas the deceleration period was prolonged at 30 degrees for MGS. PD should also show prolonged acceleration and deceleration periods in a similar manner as MSAP, if only the striatonigral pathology or parkinsonism was responsible for these changes. According to the scheme of Quaia et al. (1999), the collicular drive to accelerate contraversive saccades comes from the ipsilateral cerebellar output acting on the contralateral excitatory burst neurons (EBNs). The collicular drive to choke off the saccade speed, on the other hand, comes from the ipsilateral cerebellar input reaching the contralateral inhibitory burst neurons (IBNs). Theoretically, if both the cerebellar outputs to the IBNs and EBNs were affected in MSAP, this would result both in the increased acceleration and deceleration periods. Meanwhile, if the output from the ipsilateral cerebellar input to the contralateral IBNs were preferentially affected in PD, the deceleration period would be selectively prolonged with no change in the acceleration period. However, there is no evidence of such differential effects of cerebellar output in PD and MSAP.

The reduced velocity and hypometria seen in all types of saccades in parkinsonism may result from this SC inhibition by the basal ganglia, leading to smaller directional drive signals arriving at the brainstem saccade generator from the SC (Figs. 3–5, Hikosaka et al., 2000; Machado and Rafal, 2004). However, since the brainstem saccade generator function and accelerating signals from the cerebellar output pathway are considered to be largely preserved in PD, saccades generated will have a normal peak velocity, if its amplitude is taken into consideration. In contrast, in MSAP patients, in addition to the excessive SC suppression by the basal ganglia, both the function of the brainstem saccade generator and cerebellar input into the generator, especially to the EBNs, may be affected, and the accelerating drive for saccades may be much more reduced than in PD.

In contrast, the deceleration period of PD was significantly prolonged compared with normal subjects, though to a slightly lesser extent than in MSAP patients. Since the cerebellum and its output are expected to be largely spared in PD patients and the deceleration period deteriorated with the disease progression and increased reliably with the severity of parkinsonism, the underlying basal ganglia dysfunction may be one major cause of this, although impaired cortical processing for saccades could also contribute at later stages of the disease.

The deceleration period of PD patients was comparable to normal subjects for smaller saccades (5–10 degrees), but the difference grew longer with larger saccades, even catching up with that of MSAP patients at larger target eccentricities (Fig. 5C; Supplementary Table 1). The reason why the deceleration period increased only at large target eccentricities may be speculated upon by considering the velocity profiles at different target

eccentricities. Animal studies have shown that, for generating small amplitude saccades to a visually presented target, the two saccade signals, accelerating and decelerating, exhibit substantial overlap in time, with the activity of decelerating signals starting even before saccades onset (Buzunov et al., 2013). As saccade amplitude increases, the decelerating signals tend to occur later and the accelerating signals would largely precede the decelerating signal, with a smaller temporal overlap between them (Fuchs et al., 1993; Ohtsuka and Noda, 1991; Dean, 1995; Buzunov et al., 2013). In addition, looking at the velocity profile of saccades, the amplitude was mainly adjusted by the deceleration period for larger amplitude saccades (20–30 degrees); both the acceleration period and peak velocity increased progressively up to target eccentricities of 5–20 degrees, but at 30 degree, the acceleration period and peak velocity tended to saturate while the deceleration period now became longer. The function of the basal ganglia may be to engage the deceleration signal effectively when aiming for a target of larger eccentricity, which may be compromised in basal ganglia disorders such as PD and MSAP.

Besides functional interactions between the basal ganglia and cerebellar inputs at the thalamic and the cortical level (Alexander et al., 1986; Pelzer et al. 2017; Hintzen et al., 2018), novel bidirectional communications have been recently found between the basal ganglia and the cerebellum (Hoshi et al., 2005; Bostan et al., 2010); while the subthalamic nucleus (STN) of the basal ganglia has a substantial disynaptic projection via the pontine nucleus, input stage of the cerebellar processing, to the cerebellar cortex, whereas the deep cerebellar nuclei projects disynaptically via the thalamus to the striatum, input stage of the basal ganglia processing. Through these communications, abnormal signals from the basal ganglia in PD and MSAP may influence cerebellar function, leading to abnormal function of the cerebellar output tract, as evidenced by the increased metabolic cerebellar activity in individuals with PD (Krack et al., 1997). This in turn may cause a suboptimal modulation of the cerebellar output, leading to the prolonged and variable deceleration period in PD. In MSAP, the input stage of the cerebellar processing, the pontine nuclei, may also be affected leading to the changes in acceleration periods as well.

The changes of velocity profile was affected more severely for MGS than for VGS (Fig. 1). In Parkinsonism, MGS, a voluntary saccade, is more affected than VGS, a reflexive or reactive saccade. This may be partly because the basal ganglia are directly involved in the initiation of MGS, whereas VGS is subserved by many other redundant oculomotor pathways (Hikosaka et al., 2000; Terao et al., 2011; Pierrot-Deseilligny et al., 1991). Additionally, the effects of SC inhibition by muscimol injection or by SNr stimulation are much larger on MGS than on VGS (Hikosaka and Wurtz, 1985a, b; Liu and Basso, 2008).

### 4.4. Limitations of the study

One limitation of the present study was that dopaminergic and related medications were used appreciably more in PD and medications are increased with increasing disease severity. While dopaminergic drugs could not be discontinued for clinical and ethical reasons, the effect of the drugs will be an issue for future studies as in our previous study regarding de novo PD patients (Yugeta et al., 2008). Furthermore, in addition to dopaminergic drugs, some MSA patients were on medication targeting autonomic dysfunction, which could also affect saccade parameters. Another limitation is that the number of MSAP patients in this study was rather small, which may be an issue given that pathological confirmation was not made in this study. Finally, the use of EOG recording necessitated the use of low-pass filter at 20 Hz, since EOG was recorded by a direct current (DC) amplifier and were subject to low frequency noise which made the baseline to shift slowly as time went by even in one trial. Due to the filtering, low frequency components of the velocity profile would have been removed (Terao et al., 2018), thus reducing the actual peak velocity slightly. However, the same filtering was done for all the subject groups, so the relative amplitude and velocity comparison would be preserved.

## 5. Conclusions

In summary, saccades in MSAP were characterized both by prolonged acceleration and deceleration periods with a reduced peak velocity. In contrast, the velocity profile of PD patients was characterized predominantly by a prolonged deceleration period. The velocity profile of MSAP can be explained by the dysfunction of cerebellar acceleration and deceleration functions derived from the cerebellum, and the altered function of the brainstem saccade generator was postulated. Alternatively, the changes in velocity profile may be because the basal ganglia excessively inhibits the SC burst neurons, both in MSAP and PD. Whatever the underlying pathophysiology, the differential changes in saccade velocity profiles of MSAP and PD may serve as a potential measure for the disease progression as well as for assessing the functional improvement through saccades when clinical treatment for MSAP patients becomes available in the future.

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## Declaration of Competing Interest

None.

## Appendix A. Supplementary material

**Supplementary Data.** In addition, the raw data for the EOG traces in figure 1AB of each trial are given as supplementary excel files (NC_VGS_waveform_rawdata2, NC_MGS_waveform_rawdata2, PD_VGS_waveform_rawdata2, PD_MGS_waveform_rawdata2,

MSAP_VGS_waveform_rawdata2, MSAP_MGS_waveform_rawdata2). Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2019.09.004.

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