Voluntary, spontaneous and reflex blinking in patients with clinically probable progressive supranuclear palsy

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Patients with progressive supranuclear palsy (PSP) often have blinking abnormalities. In this study we examined the kinematic features of voluntary, spontaneous and reflex blinking in 11 patients with PSP and healthy control subjects. Patients were asked to blink voluntarily as fast as possible; spontaneous blinking was recorded during two 60 s rest periods; reflex blinking was evoked by electrical stimulation of the supraorbital nerve. Eyelid movements were recorded with the SMART analyzer motion system. During voluntary blinking the closing and opening phases lasted longer in patients than in healthy subjects. Furthermore, the peak velocity of the closing phase of voluntary blinking was lower in patients than healthy subjects. During spontaneous blinking the blink rate was markedly lower in patients than in control subjects. Patient’s recordings also showed kinematic abnormalities of spontaneous (reduced peak velocity of both closing and opening phases) and reflex (reduced peak velocity and increased duration of the opening phase) blinking. Recordings during reflex blinking disclosed an enhanced excitability of the interneuronal pool mediating the closing and opening blink phases. Finally, the pause, a neurophysiological marker of the switching processes between the closing and opening phases, was prolonged in all the three types of blinking. The abnormal kinematic variables correlated with patients’ clinical and kinematic features. Abnormal voluntary, spontaneous and reflex blinking in patients with PSP reflects the widespread cortical, subcortical and brainstem degeneration related to this disease.

Keywords: progressive supranuclear palsy; blinking; movement control

Abbreviations: ANOVA = analysis of variance; ISI = interstimulus interval; MRI = magnetic resonance imaging; NINDS-SPSP = National Institute of Neurological Disorders and Stroke-society for progressive supranuclear palsy; PSP = progressive supranuclear palsy; PSPRS = progressive supranuclear palsy rating score; ROC = receiver operating characteristic

Introduction

Eye blinking consists of sequential closing and opening eyelid movements mediated by orbicularis oculi and levator palpebrae superioris muscle activation. Blinks can be performed voluntarily, spontaneously and reflexly (VanderWerf et al., 2003). The neural control of the antagonistic interaction between orbicularis oculi and levator palpebrae superioris muscles for the closing and opening phases and the pause between them, during each type of blinking, is distributed on partially overlapping circuits in cortical, subcortical and brainstem structures (Schmidtke and Büttner-Ennever, 1992; Bour et al., 2000). Patients with progressive supranuclear palsy (PSP), manifest severe clinical disturbances of blinking and lid ‘postural’ maintenance (Esteban et al., 2004). The common clinical abnormalities of blinking include blepharospasm, blepharoclysis, lid retraction, lid opening and closing apraxia and a marked reduction in the rate of spontaneous blinking (Karson et al., 1984; Golbe et al., 1989; Grandas and Esteban, 1994; Burn and Lees, 2002).

In patients with PSP blinking has been addressed only in few neurophysiological studies. The auditory blink reflex has been reported to be absent or reduced (Vidailhet et al., 1992; Kofler et al., 2001; Williams et al., 2008). In a study using the paired shock technique to investigate the excitability of brainstem interneurons mediating the blink reflex, Valls-Sole et al. (1997) described enhanced excitability of this interneuronal pool, whereas Sommer et al. (2001) found only a non-significant trend toward excitability enhancement.
To date no information is available on the kinematic features of voluntary blinking in PSP. Such investigations would allow us to see whether the clinically well-recognized trunk and limb bradykinesia, i.e. the slowness of voluntary movements present in patients with PSP (Steele et al., 1964; Burn and Lees, 2002), is also present during voluntary eyelid movements. In addition, although some clinical and neurophysiological information is available on spontaneous and reflex blinking in patients with PSP, the kinematic features of these two types of blinking have never been studied in this disease. Nor is information available on alternating between the closing and opening phases during the three types of blinking. Finally, there are no data on possible correlations between neurophysiological abnormalities of blinking and clinical scores on disease severity, nor is it known whether the abnormal kinematic variables of blinking have a role as diagnostic tools.

In this study, we therefore analysed the kinematic features of the closing and opening phases of voluntary, spontaneous and reflex blinking, in patients with PSP and healthy controls. To clarify whether the enhanced excitability of brainstem interneurons is a neurophysiological feature of PSP we analysed the recovery cycle of the kinematic features of reflex blinking with the paired shock technique. The kinematic analysis allowed us to investigate the opening phase of blinking non-invasively (Agostino et al., 2008) thus avoiding needle electromyographic recording from the levator palpebrae superioris muscle (Aramideh et al., 1994). It also allowed us to measure the pause between the opening and closing blink phases, a variable that reflects the switching process mediating these sequential movements. To see whether possible abnormalities in the three types of eyelid movements coexist, we examined the three types of blinking in the same group of patients in a single experimental session. We also determined the possible correlations between patients’ clinical features and neurophysiological data. Finally, to assess their diagnostic performance and accuracy in distinguishing patients from healthy controls, we analysed the receiver operating characteristic (ROC) curves for each abnormal kinematic variable.

### Patients and methods

We studied 11 patients with a clinical diagnosis of probable PSP (mean age ± 1SD: 66.6 ± 4.7 years, other clinical features are reported in Table 1), and 10 age-matched healthy controls (mean age ± 1SD: 65 ± 9 years). Patients with PSP were studied only while they were on their therapeutic regimen. Of the 11 patients studied, 7 were receiving L-dopa (together with dopa decarboxylase inhibitors) alone or combined with other types of drugs. One patient was taking amantadine only, another patient was taking citalopram only and two patients were without treatment (Table 1). Patients with Mini-Mental-State-Examination scores lower than 21/30 were excluded from the study. All participants gave their informed consent and the study was approved by the local ethical committee. PSP was diagnosed according to the National Institute of Neurological Disorders and Stroke-Society for PSP (NINDS-SPSP) clinical research criteria (Litvan et al., 1996). The severity of PSP was rated according to the clinical rating scale (PSPRS) proposed by Golbe et al. (2007). Magnetic resonance imaging (MRI) scans, obtained at various times during the two years before the study to make the differential diagnosis between PSP and other disorders, showed that of the 11 patients enrolled in the study 8 had various degrees of brainstem, rostral midbrain, basal ganglia and frontal lobe involvement and 3 had mild generalized brain atrophy.

Voluntary, spontaneous and reflex blinking were studied as previously detailed (Agostino et al., 2008) in a single experimental session. Patients and control subjects were comfortably seated on a chair, with their head placed in a head holder and their forehead fixed, looking straight-forward at a fixation point. The three types of blinking were examined in randomized order. To elicit voluntary blinking, the subjects were instructed to blink as fast as possible after a verbal command. After a brief practice session, 15 trials were collected with an inter-trial interval of about 5 s. Spontaneous blinking was recorded while the subjects were asked to remain relaxed and look at a fixation point. Two 60 s epochs were recorded. This duration was chosen because previous findings in two large cohorts of normal subjects and patients with movement disorders showed that a 60 s recording is long enough to provide reliable results (Deuschl and Godde, 1998). Reflex blinking was recorded after a single electrical stimulus (square wave pulse, duration: 0.2 ms) applied transcutaneously to the right supraorbital nerve at the supraorbital notch. To evoke stable blink reflex responses, we used an intensity of about six times the sensory threshold determined as the lowest stimulus able to provide reliable results (Deuschl and Godde, 1998).

### Patients and methods

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th>Age (year)</th>
<th>Disease duration (yr)</th>
<th>PSPRS (total score)</th>
<th>Treatment (daily dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>66</td>
<td>5</td>
<td>47</td>
<td>L-dopa (600 mg) + amantadine (150 mg)</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>71</td>
<td>4</td>
<td>56</td>
<td>Citalopram (20 mg)</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>68</td>
<td>8</td>
<td>46</td>
<td>L-dopa (400 mg)</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>69</td>
<td>5</td>
<td>57</td>
<td>Amantadine (200 mg)</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>70</td>
<td>3</td>
<td>54</td>
<td>L-dopa (600 mg) + amitriptyline (20 mg)</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>73</td>
<td>10</td>
<td>53</td>
<td>L-dopa (600 mg) + amantadine (300 mg)</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>68</td>
<td>2</td>
<td>25</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>64</td>
<td>3</td>
<td>33</td>
<td>L-dopa (300 mg)</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>56</td>
<td>3</td>
<td>44</td>
<td>L-dopa (300 mg) + escitalopram (20 mg)</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>66</td>
<td>4</td>
<td>45</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>Female</td>
<td>62</td>
<td>1</td>
<td>38</td>
<td>L-dopa (100 mg)</td>
</tr>
</tbody>
</table>

The range of PSPRS total score is from 0 to 100.
the reflective marker. Before the automatic analysis, an examiner followed by a brief recording to measure the static position of blinking was recorded, the eyelid movements were preceded and ended by the complete blink movement, when each type of blink oscillations. For spontaneous blinking, we measured the pause, i.e. the time elapsing between the end of the closing phase and the beginning of the opening phase (patients and controls) for each kinematic variable. Data for voluntary, spontaneous and reflex blinking were analysed separately. To analyse the recovery cycles for amplitude and peak velocity during the closing and opening phases, we used a two-way ANOVA for repeated measures with the between group factor GROUP (patients and controls) and the within group factor ISI (200 s and 600 ms). Tukey honest significant difference test was used for post hoc analysis. Possible correlations between each abnormal kinematic variable were evaluated with the Pearson product moment correlation analysis. To study the correlations between abnormal kinematic variables and clinical data such as PSPRS scores, disease duration L-dopa and amantadine total daily dose, we performed a multiple regression analysis. For each abnormal kinematic variable (dependent variable), we determined the significance and the coefficient of determination ($R^2$) of the multiple correlation. $R^2$ (values ranging from 0 to 1) is the proportion of the variation in the dependent variable explained by the regression model and is a measure of the goodness of fit of the model. For each multiple correlation, we then determined the significance of the contribution of each clinical variable analyzed (independent variables). Because Sforza et al. (2008) have recently shown that spontaneous blinking in healthy subjects is highly variable in amplitude, to measure the intra-subject variability for each subject and for each kinematic variable of 15 responses were collected with an inter-trial interval of about 40-60 s. We also studied the excitability of reflex by determining its recovery cycle. To do so we used the paired shock technique by delivering a first conditioning stimulus followed at 500 and 1 000-ms interstimulus intervals (ISIs) by a second test stimulus (Kimura, 1973). We did not use shorter ISIs because a preliminary observation of the raw data, showed that in patients with PSP kinematic measurement of the complete reflex blinking (duration of the closing phase plus duration of the pause and duration of the opening phase) lasted about 400 ms (see also Table 2). The two ISIs were presented in randomized order. Five trials were collected for each ISI with an inter-trial interval of about 40–60 s (Berardelli et al., 1985a, b; Agostino et al., 2008).

Voluntary, spontaneous and reflex blinking were recorded with the SMART analyzer motion system (BTS, Milan, Italy). This system comprises three infrared cameras (120 Hz sampling rate) able to follow the displacement, in the 3D space, of a reflective marker taped on the right-upper eyelid. To check the beginning and end of the complete blink movement, when each type of blinking was recorded, the eyelid movements were preceded and followed by a brief recording to measure the static position of the reflective marker. Before the automatic analysis, an examiner visually inspected the traces to identify the very rare traces (<1% of traces for each subject) without a complete blink movement recording and excluded them from subsequent analysis. To measure the entire duration of a single blink the examiner put two markers, one before the closing phase of blinking started and one after the opening phase ended. The interval between the two markers defined the interval during which a dedicated software measured the kinematic variables considered for the offline analysis. For the automatic analysis the software defined the beginning and end of the closing and opening blink phases when the velocity first reached or returned to 10% of the peak velocity of each phase. The procedure adopted for the end of the opening phase of the blink excluded possible contamination by blink oscillations. For all the three types of blinking, duration, peak velocity and amplitude of the closing and opening phases were measured. We also measured the pause, i.e. the time elapsing between the end of the closing phase and the beginning of the opening phase of the same blink. For spontaneous blinking, we determined the blink rate, i.e. the number of blinks in a minute (Deuschl and Goedemeier, 1998; Agostino et al., 2008). Finally, to determine the reflex blinking recovery cycle we calculated the peak velocity and amplitude, of the closing and opening phases of the response to the test stimulus as a percentage of the size of the response to the conditioning stimulus (Agostino et al., 2008).

We selected these kinematic variables because previous observations showed that the peak velocity and amplitude of the closing phase correlate positively with the size of orbicularis oculi muscle activation in response to supraorbital electric nerve stimulation (VanderWert et al., 2003). This feature allows a better comparison between electromyographic and kinematic studies on blinking excitability.

In each subject for both voluntary and reflex blinking to a single stimulus, the value of each variable was the mean of 15 trials. For the recovery cycle of the reflex blinking, the peak velocity and amplitude values for the closing and opening phases were the mean of five trials, for each ISI. For spontaneous blinking the value of each variable was the mean number of blinks recorded during the two 60-s epochs. To determine possible differences between patients and control subjects we used a one-way analysis of variance (ANOVA) with the between group factor GROUP (patients and controls) for each kinematic variable. Data for voluntary, spontaneous and reflex blinking were analysed separately. To analyse the recovery cycles for amplitude and peak velocity during the closing and opening phases, we used a two-way ANOVA for repeated measures with the between group factor GROUP (patients and controls) and the within group factor ISI (500 and 1000 ms). Tukey honest significant difference test was used for post hoc analysis. Possible correlations between each abnormal kinematic variable were evaluated with the Pearson product moment correlation analysis. To study the correlations between abnormal kinematic variables and clinical data such as PSPRS scores, disease duration L-dopa and amantadine total daily dose, we performed a multiple regression analysis. For each abnormal kinematic variable (dependent variable), we determined the significance and the coefficient of determination ($R^2$) of the multiple correlation. $R^2$ (values ranging from 0 to 1) is the proportion of the variation in the dependent variable explained by the regression model and is a measure of the goodness of fit of the model. For each multiple correlation, we then determined the significance of the contribution of each clinical variable analyzed (independent variables). Because Sforza et al. (2008) have recently shown that spontaneous blinking in healthy subjects is highly variable in amplitude, to measure the intra-subject variability for each subject and for each kinematic variable of

## Table 2: Kinematic variables for the three types of blinking in patients with PSP and healthy control subjects

<table>
<thead>
<tr>
<th>Kinematic variables</th>
<th>Voluntary blinking</th>
<th>Spontaneous blinking</th>
<th>Reflex blinking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSP</td>
<td>Controls</td>
<td>PSP</td>
</tr>
<tr>
<td>Pause</td>
<td>478.6 ± 121”</td>
<td>44.2 ± 96</td>
<td>106.2 ± 30.2”</td>
</tr>
<tr>
<td>Closing duration</td>
<td>115.5 ± 15.7”</td>
<td>70.0 ± 4.3</td>
<td>95.9 ± 15.4</td>
</tr>
<tr>
<td>Opening duration</td>
<td>342.6 ± 52.5”</td>
<td>168.5 ± 15.9</td>
<td>273.6 ± 53.5</td>
</tr>
<tr>
<td>Closing peak velocity</td>
<td>265.2 ± 24.8”</td>
<td>340.1 ± 25.4</td>
<td>132.2 ± 23”</td>
</tr>
<tr>
<td>Opening peak velocity</td>
<td>1278 ± 17.4</td>
<td>154.1 ± 10.5</td>
<td>609.9 ± 9.5”</td>
</tr>
<tr>
<td>Closing amplitude</td>
<td>11.9 ± 0.7</td>
<td>12.4 ± 0.7</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>Opening amplitude</td>
<td>10.9 ± 0.8</td>
<td>12.0 ± 0.7</td>
<td>5.3 ± 0.9</td>
</tr>
</tbody>
</table>

The pause and durations are expressed in milliseconds. Velocities are expressed in millimetre per second. Amplitudes are expressed in millimetre. Plus and minus values are means ± ISE. *Statistically significant difference between patients and healthy controls ($P \leq 0.05$ by one-way ANOVA).
spontaneous blinking we determined the coefficient of variation. Inter-group differences for the coefficient of variation were analysed with Student t-test for unpaired data. P values of \( \leq 0.05 \) were considered to indicate statistical significance.

To assess the diagnostic accuracy (sensitivity and specificity) of kinematic analysis for PSP we then constructed ROC curves. For each abnormal kinematic variable (defined on the basis of the previous ANOVA analysis) we calculated the 'cut-off value' corresponding to the highest diagnostic accuracy (minimal false-negative and false-positive results). The 'cut-off value' was the point on the ROC curve, calculated by a dedicated software, that maximizes correct classifications of pathological conditions and minimizes errors. Because 'cut-off values' depend on the different overlap in the distributions of each variable between healthy subjects and patients, cut-off values were not related to the SD. We then assessed the diagnostic sensitivity [proportion of patients with PSP in whom each kinematic variable correctly yielded abnormal findings (true positives)] and specificity [proportion of control subjects in whom each kinematic variables correctly yielded normal findings (true negatives)] (Pepe, 2003).

**Results**

**Voluntary blinking**

One-way ANOVA disclosed significant differences in kinematic variables measured during voluntary blinking between patients with PSP and healthy controls. The closing and opening blink phases both had significantly longer durations in patients than in healthy controls \([F(1,19) = 6.97, P < 0.05 \text{ and } F(1,19) = 9.28, P < 0.01]\), the closing phase had lower peak velocity \([F(1,19) = 4.48, P < 0.05]\) and the pause between the two blink phases lasted longer \([F(1,19) = 11.64, P < 0.01]\). Conversely, the closing and opening blink phases were similar in amplitude and the opening blink phase was similar in peak velocity in patients and healthy controls (Fig. 1, Table 2).

**Spontaneous blinking**

Like voluntary blinking, spontaneous blinking differed in patients with PSP and healthy controls. The mean blink rate

![Fig. 1 Velocity curves for voluntary, spontaneous and reflex blinking in patients with PSP (black line) and control subjects (grey line). The x-axis corresponds to time (ms) and the y-axis to velocity (mm/s). The first peak in the velocity trace corresponds to the peak velocity of the closing phase and the second peak to the peak velocity of the opening phase. Note the prolonged pauses for all three types of blinking in patients with PSP.](http://brain.oxfordjournals.org/)

was markedly lower in patients than in controls [mean values ± 1SE: 5.9 ± 2 blinks/min versus 22.4 ± 3.6 blinks/min; \( F(1,19) = 15.97, P < 0.01 \)]. The closing and opening blink phases both showed slower peak velocity in patients than in controls \( [F(1,19) = 4.48, P < 0.05, F(1,19) = 8.52, P < 0.01] \). The pause between the closing and opening blink phases was significantly prolonged \([F(1,19) = 8.42, P < 0.01]\). Conversely, the closing and opening phases were of similar amplitude and duration in patients and healthy controls (Fig. 1, Table 2).

The analysis of the inter-group differences in the coefficient of variation for each kinematic variable during spontaneous blinking showed that the only variable that was similar in patients and control subjects was pause duration (mean values ± 1 SE: 0.94 ± 0.12 versus 0.98 ± 0.17). The remaining kinematic variables showed greater variability in patients than in control subjects (closing duration: 0.42 ± 0.07 versus 0.17 ± 0.03; \( P < 0.01 \); opening duration: 0.62 ± 0.14 versus 0.28 ± 0.16; \( P < 0.01 \); closing peak velocity: 0.47 ± 0.08 versus 0.21 ± 0.04; \( P = 0.01 \); opening peak velocity: 0.50 ± 0.09 versus 0.27 ± 0.04; \( P = 0.05 \); closing amplitude 0.43 ± 0.07 versus 0.20 ± 0.02; \( P = 0.01 \); opening amplitude 0.50 ± 0.08 versus 0.26 ± 0.02; \( P = 0.01 \)).

### Reflex blinking

Kinematic variables during reflex blinking evoked by electrical stimuli delivered to the supraorbital nerve also differed in patients with PSP and healthy controls. In patients, the opening blink phase was significantly increased in duration \([F(1,19) = 14.52, P < 0.01]\) and exhibited decreased peak velocity \([F(1,19) = 11.49, P < 0.01]\); the pause between the two blink phases was significantly increased in duration \([F(1,19) = 6.11, P < 0.05]\). The amplitudes of both closing and opening phases as well duration and peak velocity of the closing phase were similar in patients and healthy controls (Fig. 1, Table 2).

Repeated-measure ANOVA testing the recovery cycles for the peak velocity and amplitude of the closing and opening blink reflex phases disclosed a significant effect of the between group factor GROUP \([F(4,15) = 36.93, P < 0.01]\) whereas the within group factor ISI was not significant. Nor was the interaction between the two factors GROUP and ISI significant. Post hoc analysis showed that at the 500 and 1000 ms ISIs the percentage values of the peak velocity of the closing and opening phases and of the amplitude of the opening phase were significantly greater in PSP patients than in healthy subjects \((P < 0.01)\) (Table 3).

### Correlations among the abnormal kinematic variables

Pearson product moment correlation test showed that the blink rate was inversely correlated with the duration of the pause \((r = -0.75, P < 0.05)\) and with the peak velocity of the closing phase of spontaneous blinking \((r = -0.71, P < 0.05)\). An inverse correlation was also found between the blink rate and the percentage values of the peak velocity of closing phases of the response to the test stimulus at 1000 ms \((r = -0.80, P < 0.05)\).

### Correlations between clinical and kinematic variables

Multiple regression analysis showed no significant correlation between abnormal kinematic variables for spontaneous and voluntary blinking and clinical data (PSPRS clinical score, l-dopa and amantadine total daily dose, disease duration). Conversely, the duration of the pause between the closing and opening phases of reflex blinking significantly correlated with clinical data \((R^2 = 0.90, P < 0.01)\). The only variables that contributed significantly to the correlation were PSPRS clinical score \((r = 0.66; P < 0.01)\) and l-dopa dose \((r = -0.45; P < 0.01)\), indicating that the more severe the disease the longer was the pause and the higher the l-dopa dose the shorter was the pause.

### ROC curve analysis

The ROC curve analysis showed that 6 out of 18 abnormal kinematic variables (pause during all the three types of blinking, reflex blinking opening duration, peak velocity and amplitude recovery cycles for the opening phase of reflex blinking tested at 1000 ms ISI) yielded 100% diagnostic specificity although their sensitivity ranged from 40% to 91.6%. The variable that yielded the highest specificity (100%) and highest sensitivity for PSP (91.6%) was a prolonged pause during reflex blinking. Pauses during voluntary and spontaneous blinking also had high sensitivity (81.82 and 72.73%). Only two blinking variables (the duration of the opening phase during voluntary blinking and the blink rate) had 100% sensitivity, accompanied by lower specificity (80%) for PSP than that of the

| Table 3 Recovery cycle of reflex blinking in patients with PSP and healthy control subjects |
|---|---|---|---|---|
| Interstimulus interval | 500 ms | 1000 ms |
| Kinematic variables | PSP | Controls | PSP | Controls |
| Closing peak velocity | 6.5 ± 8.6* | 96 ± 6.7 | 90.1 ± 91* | 81.0 ± 4.4 |
| Opening peak velocity | 89.5 ± 9.9* | 61.0 ± 8.6 | 107.4 ± 6.5* | 83.7 ± 5.7 |
| Closing amplitude | 60.9 ± 9.5 | 54.3 ± 91 | 76.1 ± 79 | 770 ± 3.2 |
| Opening amplitude | 71.4 ± 10.5* | 53.7 ± 11.3 | 98.0 ± 99* | 75.8 ± 3.9 |

Values (mean ± ISE) are expressed as a percentage of the test stimulus with respect to the conditioning stimulus. Statistically significant difference between patients and healthy controls \((P < 0.05)\) by two-way ANOVA for repeated measures.
Discussion

In this article, we have now shown for the first time that in patients with PSP voluntary, spontaneous and reflex blinking all show abnormal kinematic features. Our article also provides evidence that in all three types of blinking the switching process between the closing and opening phases is impaired. Blink abnormalities correlated with each other and with patients’ clinical features, namely PSPRS score and L-Dopa daily dose, and some abnormal kinematic variables achieved <100% specificity (Table 4).

The first new finding in our study is that the closing and opening phases during voluntary blinking lasted longer in patients with PSP than in healthy controls. The closing phase during voluntary blinking also had reduced peak velocity. These findings provide evidence of bradykinetic voluntary blinking in patients with PSP. The reciprocal activation of the orbicularis oculi and levator palpebrae superiorius muscles during voluntary blinking is controlled by cortical areas on the frontal lobe convexity (Esteban et al., 2004). As suggested by neuroradiological and neurophysiological studies in healthy humans (Montagna and Zucconi, 1984; Kaneko et al., 2004; Sohn et al., 2004), in addition to the dorsolateral frontal areas, other areas that might play a role in controlling voluntary blinking include the mesial frontal region, and in particular the supplementary motor area. Bradykinesia of voluntary blinking may therefore result from neurodegenerative processes directly involving the frontal areas (Daniel et al., 1995; Morris et al., 2002). Bradykinesia of voluntary blinking may also be due to neuronal loss and gliosis affecting the striatum, pallidum, substantia nigra and subthalamic nucleus (Daniel et al., 1995; Morris et al., 2002) resulting in functional deafferentation of the frontal lobe areas involved in the so-called basal ganglia motor loop (DeLong and Wichmann, 2007). Supporting the pathological findings of widespread cortical and subcortical degeneration in patients with PSP (Daniel et al., 1995; Morris et al., 2002), some imaging evidence, obtained with positron emission tomography, shows glucose hypometabolism in the midline frontal regions and in caudate nucleus of patients with PSP (Juh et al., 2004; Eckert et al., 2005). Further studies with voxel-based morphometry and diffusion tensor imaging confirmed severe structural changes involving cortical and subcortical brain regions as well as neurodegeneration involving specific fibre tracts such as the cortico-bulbar tracts (Padovani et al., 2006). Since in a previous study in patients with Parkinson’s disease (Agostino et al., 2008), we found that the closing and opening phases of voluntary blinking had normal duration and peak velocity the kinematic blink features during voluntary blinking that distinguish patients with PSP from those with Parkinson’s disease are the prolonged duration of the closing and opening phases and the reduced peak velocity of the closing phase. The opening phase duration during voluntary blinking was highly sensitive for PSP although its specificity was 80%.

As well as confirming the markedly reduced spontaneous blink rate in patients with PSP (Karson et al., 1984), our study provides previously unreported kinematic information that extends current knowledge on neural control of spontaneous blinking. Our patients had lower peak velocities in the two blink phases than healthy subjects. The analysis of the coefficient of variation showed that the duration, peak velocity and amplitude of the closing and opening phases during spontaneous blinking were significantly more variable in patients with PSP than in control subjects.
The blink rate is a good indicator of central dopaminergic activity (Karson, 1983) and parkinsonian syndromes, including PSP, are characterized by a marked reduction in central dopaminergic tone (Karson et al., 1984). Hence, the pronounced reduction in the blink rate observed in our patients with PSP presumably depends on reduced central dopaminergic activity. The blink rate we found in our patients with PSP was lower than that previously observed in patients with Parkinson’s disease (Korosec et al., 2006; Agostino et al., 2008). This finding confirms the original results by Karson et al. (1984) and those of Altiparmak et al. (2006). Interestingly, the blink rate was highly sensitive (100%) for PSP although the specificity was lower (80%). The reduced peak velocities of the closing and opening phases of spontaneous blinking we found in this kinematic study on patients with PSP could also reflect the reduced central dopaminergic activity on the cerebral structures generating spontaneous blinking. The presumed relationship between peak velocities of the closing and opening phases of spontaneous blinking and central dopaminergic activity agrees with kinematic data during spontaneous blinking in patients with Parkinson’s disease, showing decreased peak velocity and amplitude during the closing and opening phases of spontaneous blinking (Kaneko et al., 2001; Agostino et al., 2008). To our knowledge, cerebral structures generating spontaneous blinking have not yet been identified. Although some results from functional MRI and electrooculogram measurement suggest a role of the mesial frontal region (Yoon et al., 2005), other studies showing the absence of movement-related potentials imply otherwise (Montagna and Zucconi, 1984; Kaneko et al., 2004).

Unlike voluntary and spontaneous blinking, the kinematic analysis of reflex blinking showed that the opening phase, but not the closing phase, lasted longer in patients with PSP than in healthy subjects and its peak velocity was reduced. Our findings on the impaired opening phase agree with the hypothesis that PSP causes excessive inhibition of the levator palpebrae superioris muscle (Esteban et al., 2004). The levator palpebrae superioris muscle is innervated by motoneurons arising from the central caudal nucleus situated in midbrain within the oculomotor nucleus (Schmidtke and Büttner-Ennever, 1992). Although the structures involved in generating tonic central caudal nucleus activity are still unknown, several observations suggest a role of the periaqueductal grey matter overlying the central caudal nucleus, considered a hypothetical site for pre-motor interactions of signals controlling the levator palpebrae superioris muscle motoneuronal pool (Schmidtke and Büttner-Ennever, 1992). Support for excessive levator palpebrae superioris muscle inhibition comes from quantitative MRI analysis showing pronounced brainstem atrophy and reduction of the rostral midbrain area in PSP (Kato et al., 2003; Oba et al., 2005; Slowinski et al., 2008). In their study investigating the relationship between MRI features and pathological brainstem findings in patients with PSP, Aiba et al. (1997) showed that the tegmental and tectal atrophy, and aqueductal dilatation detected with T1-weighted images at midbrain level were histologically consistent with atrophy of several midbrain structures including periaqueductal gray matter. In addition to brainstem abnormalities, the cerebellum and the superior cerebellar peduncle also degenerate in PSP (Kataoka et al., 2008). Further evidence confirming the role of the cerebellum in modulating the trigeminal blink reflex comes from the study by Chen and Evinger (2006) showing that the nucleus interpositus intervenes in enhancing the excitability of the levator palpebrae superioris muscle.

When we studied reflex blinking with the paired shock technique to clarify whether the enhanced excitability of brainstem interneurons is a neurophysiological feature of PSP we found, in agreement with Valls-Solé et al. (1997), that patients with PSP had enhanced excitability of the neural circuits mediating the closing phase of reflex blinking. In addition, we show for the first time that also the excitability of the neural circuitry mediating the opening phase of reflex blinking is enhanced. Our findings on the closing phase of blinking agree with the current hypothesis that the basal ganglia exert an inhibitory descending control on the excitability of the brainstem interneurons mediating the blink reflex via the colliculus superior, the nucleus raphe magnus and the spinal trigeminal complex (Basso and Evinger, 1996; Basso et al., 1996). In PSP the neuronal loss in subcortical structures and especially in the globus pallidus, subthalamic nucleus and substantia nigra (Daniel et al., 1995; Morris et al., 2002) may therefore produce an abnormal basal ganglia output that disinhibits brainstem reflexes. Why patients with PSP also exhibit enhanced excitability of the neural circuitry mediating the opening phase of reflex blinking is unclear. Since the recovery cycle studied by means of paired shock technique is a function of the number of interneurons mediating the closing phase of the reflex blinking (Kimura, 1973), a polysynaptic interneuronal network for the opening phase of the blink reflex might also exist and eventually mediate levator palpebrae superioris muscle activation in response to trigeminal stimulation. The last original finding of our study is that our patients with PSP had a distinctive lengthening of the pause during all the three types of blinking. The switching between the closing and opening phases of blinking reflects the fine coordination of the timing and reciprocity of orbicularis oculi and levator palpebrae superioris muscle activation (Schmidtke and Büttner-Ennever, 1992). In healthy subjects, during each type of blinking the overall duration of orbicularis oculi muscle activation is shorter than the levator palpebrae superioris muscle inhibition (Esteban et al., 2004). In patients with PSP, the lengthening of the pause during all the three types of blinking might therefore depend on the partial co-contraction of the antagonist orbicularis oculi and levator palpebrae superioris muscles. Since the pause is lengthened during reflex blinking the...
brainstem damage related to PSP may have a predominant role in determining this finding but also in lengthening the pause during voluntary and spontaneous blinking mediated by inputs starting from cortical and subcortical structures but converging on the brainstem itself. In a previous study on patients with Parkinson’s disease, we nevertheless showed that the pause between the closing and opening phases of blinking is abnormally long only during voluntary blinking and interpreted this finding as reflecting the selective abnormal function of mesial cortical areas in the frontal lobe (Agostino et al., 2008), a major target of the basal ganglia motor loop (DeLong and Wichmann, 2007). In this study, the ROC curve analysis showed that the pause duration of voluntary spontaneous and reflex blinking was highly specific (100% for all the three types of blinking) for PSP and the pause duration during reflex blinking had the highest sensitivity (91.6%).

The correlation analysis showed that the blink rate correlated inversely with some of the kinematic abnormalities of spontaneous blinking. For example, the lower the blink rate the longer were the pause duration and the higher the peak velocity of the closing phase. These findings suggest that in PSP the abnormalities in the blink rate and the abnormal kinematics during spontaneous blinking possibly arise through similar pathophysiological mechanisms. In addition, we found that the lower the blink rate the higher was the excitability of the interneuronal pool mediating the closing phase. Hence the reduced dopaminergic tone concurrently influences the blink rate and the recovery cycle for the closing phase of reflex blinking. We also found that the PSPRS score correlated positively with pause duration during reflex blinking. This finding suggests that this kinematic variable of reflex blinking may be a useful quantitative marker for monitoring PSP progression or the effect of new therapeutic strategies. In addition, pause duration during reflex blinking could also be an effective tool in the diagnosis of PSP since it was a highly sensitive and specific variable. Furthermore, because a previous study has shown that the duration of the pause during reflex blinking is normal in patients with Parkinson’s disease (Agostino et al., 2008), pause duration can differentiate PSP from Parkinson’s disease.

In our study, we found marked abnormalities in the three types of blinking, even though most patients with PSP were on dopaminergic medication. Interestingly, we found that the higher the L-dopa dose the shorter was the pause during reflex blinking. We therefore conjecture that blinking abnormalities might be even more evident when patients are off dopaminergic drugs.

In conclusion, voluntary, spontaneous and reflex blinking all show abnormal kinematic features in patients with PSP. Voluntary blinking is markedly bradykinetic. Hence bradykinesia, a cardinal sign of patients with PSP, manifests also in the upper face. In addition to a markedly low rate, spontaneous blinking is characterized by a low peak velocity of the closing and opening phases. During reflex blinking, patients perform the opening phase slowly and the excitability of the closing and opening phases is enhanced. As they do during voluntary blinking, during spontaneous and reflex blinking patients with PSP also exhibit a lengthy pause between the closing and opening phases. Since pause duration during reflex blinking had a significant correlation with PSP severity and duration and yielded high sensitivity and specificity it could play a useful role in establishing the diagnosis of PSP and evaluating new therapies. Finally, our study showed that among other things distinguishing patients with PSP from those with Parkinson’s disease (Agostino et al., 2008) are more marked bradykinesia of voluntary blinking, a more pronounced reduction in the blink rate, and more abnormal switching between the closing and opening phase not only during voluntary blinking but also during spontaneous and reflex blinking. Some kinematic features of the opening phase are also impaired during all the three types of blinking. Our kinematic findings suggest that in patients with PSP the diffuse pathological degeneration of cortical, subcortical and brainstem structures affect the neural systems controlling all the three types of blinking.

References


