Eyelid Movements: Behavioral Studies of Blinking in Humans Under Different Stimulus Conditions

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VanderWerf, Frans, Peter Brassinga, Dik Reits, Majid Aramideh, and Bram Ongerboer de Visser. Eyelid movements: behavioral studies of blinking in humans under different stimulus conditions. *J Neurophysiol* 89: 2784–2796, 2003. First published December 27, 2002; 10.1152/jn.00557.2002. The kinematics and neurophysiological aspects of eyelid movements were examined during spontaneous, voluntary, air puff, and electrically induced blinking in healthy human subjects, using the direct magnetic search coil technique simultaneously with electromyographic recording of the orbicularis oculi muscles (OO-EMG). For OO-EMG recordings, surface electrodes were attached to the lower eyelids. To measure the vertical lid displacement, a search coil with a diameter of 3 mm was placed 1 mm from the rim on the upper eyelid on a marked position. Blink registrations were performed from the zero position and from 28 randomly chosen positions. Blinks elicited by electrical stimulation of the supraorbital nerve had shortest duration and were least variable. In contrast, spontaneous blinks had longer duration and greater variability. Blinks induced by air puff had a slightly longer duration and similar variability as electrically induced blinks. There was a correlation between the maximal down phase amplitude and the integrated OO-EMG. Blink duration and maximal down phase amplitude were affected by eye position. Eyes positioned 30° above horizontal displayed the shortest down phase duration and the largest maximal down phase amplitude and velocity. At 30° below horizontal, blinks had the longest total duration, the longest down phase duration, and the lowest maximal down phase amplitude and velocity. The simultaneously recorded integrated OO-EMG was largest in the 30° downward position. In four subjects, the average blinking data showed a linear relation between eye position and OO-EMG, maximal down phase amplitude, and maximal downward velocity.

INTRODUCTION

In all types of eyelid movements, two skeletal eyelid muscles, the levator palpebrae superioris (LPS) and the orbicularis oculi (OO) muscles, and two smooth muscles, the superior tarsal and inferior tarsal muscles (Müller's), are involved. In blinking, LPS and OO muscles act antagonistically (Aramideh et al. 1994, 2002; Evinger et al. 1991; Kugelberg 1952). Motoneurons in the central caudal subnucleus of the oculomotor complex innervate the LPS muscle, while OO muscle fibers receive their innervation from the intermediate zone of the facial nucleus (Becker and Fuchs 1988; Schmidtke and Bütt-

ner-Ennever 1992; VanderWerf et al. 1997, 1998). During a blink, LPS motoneurons stop firing briefly, while OO motoneurones produce a short burst, activating OO muscle fibers and causing a rapid lowering of the upper eyelid (Aramideh et al. 2002; Trigo et al. 1999). Stereotyped eye movements during blinking (Bour et al. 2000; Collewijn et al. 1985) accompany eyelid movements. At the onset of a blink, the eye rotates normally from the initial position nasally downward; the extent of eye rotation depends on the initial eye position (Bour et al. 2000).

The blink reflex can be initiated by an electrical stimulation of the supraorbital (SO) nerve, an air puff, a light flash, or a sound (Esteban 1999; Kugelberg 1952; Pelligrini et al. 1995; Powers et al. 1997). The blink reflex elicited by electrical SO nerve stimulation consists of an early ipsilateral response (R_1) and a late bilateral response (R_2) (Aramideh et al. 1994; Kimura 1970; Ongerboer de Visser et al. 1978). SO nerve-evoked reflex blinks have been studied by a number of investigators (Cruccu and Deuschl 2000; Evinger et al. 1991; Hammond et al.1996; Kugelberg 1952; Ongerboer de Visser 1998; Wouters et al. 1995), but only Bour et al. (2000), Evinger et al. (1991, 2001), and Snow and Frith (1989) recorded simultaneously OO muscle activity and eyelid movements. Spontaneous blinks have been studied most extensively in the human (Evinger et al. 1984, 1991; Guitton et al. 1991; Niida et al. 1987; Stava et al. 1994; Sun et al. 1997) and in the cat (Gruart et al. 1995). Kinematic studies of voluntary blinks (Collewijn et al. 1985; Gittins et al. 1999; Guitton et al. 1991; Niida et al. 1987; Sun et al. 1997) have been studied most extensively, while studies of concomitant lid movements and OO-EMG recordings have not been performed in the human.

Air puff studies have been performed in humans (Collewijn et al. 1985; Evinger et al. 1984; Guitton et al. 1991), cats (Gruart et al. 1995; Trigo et al. 1999), guinea pigs, and rabbits (Evinger et al. 1984; Gruart et al. 2000). The kinematics of air puff–induced eyelid movements were studied by applying the air puff to the periorbital region (Evinger et al. 1994); so far, simultaneous EMG and eyelid movement recordings of air puff–induced blinks have not been performed in humans.

Other authors have studied blink kinematics, alone or together with eyelid muscle activity induced by glabellar tap or

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direct corneal stimulation, or evoked by electrical stimulation of the ophthalmic branch of the facial nerve (Cruccu and Deuschl 2000; Esteban and Salinero 1979; Ongerboer de Visser et al. 1977; Snow and Frith 1989).

Neurophysiological and neuroanatomical data suggest that the trigeminal blinking system plays a role in blink adaptation (Aramideh et al. 2001; Kimura 1972; Pelligrini and Evinger 1995; Pelligrini et al. 1995; Peshori et al. 2001; Powers et al. 1997; VanderWerf et al. 1997; Van Ham and Yeo 1996) and probably in eye/eyelid coordination (Goossens and Van Opstal 2000a,b; Ndiaye et al. 2002; VanderWerf et al. 2002).

Because the up phase duration of a blink is hard to define, a procedure to estimate its duration has been developed. Based on this new method, air puff and electrically induced reflex blinks and voluntary and spontaneous blinks were systematically examined under eye fixation and under various eye positions using the magnetic search coil technique. To provide detailed insight in eyelid kinematics, the vertical displacement of different types of blinking were recorded under various eye positions, with emphasis on the integrated OO-EMG, the simultaneous maximal down phase amplitude, and maximal downward velocity of the eyelid.

METHODS

Subjects

Five healthy male (M1–M5) and one healthy female (F1) subjects, 22–59 yr of age, participated in this study. Test protocols and ethics committee approval was in accordance with the tenets of the Declaration of Helsinki.

Eyelid movement recordings

Movements of the right eyelids were measured with the magnetic search coil technique (Remmel 1984). The subject's head was positioned in the center of a magnetic alternating field (75 × 75 × 75 cm) of 30 and 45 kHz, for the horizontal and vertical eyelid movement, respectively.

As the coil on the eyelid slides over the curved surface of the eye, the upper eyelid contains rotation as well as vertical translation (Wouters et al.1995). Calibration showed that the rotation correlates with the actual eyelid movement in the vertical direction. The system detected lid rotations as small as 0.25°, which is an equivalent to a vertical lid motion of 0.06 mm (Remmel 1984).

A coil (2.2 mm diam, 20 turns, 20 mg) of copper wire (0.1 mm diam), specially constructed for these experiments, was used. The coil was taped on the eyelid approximately 1 mm from the margin of the upper eyelid above the pupil when the subject looked in the straight-forward position. Subjects became unaware of the coil shortly after application.

The onset of the down phase of lid movement was defined when the recording started to deviate more than 0.2° from the zero position. The termination of the blink up phase was defined as the point where the up phase amplitude was 95% of the maximal down phase amplitude. The up phase time duration (T-up-phase) is defined as the termination of the blink minus the time the maximal deviation (Tmax) was reached (Fig. 1).

EMG recording

For the OO-EMG recording, two 10-mm-diam Ag/AgCl surface electrodes were used. One was placed 10 mm laterally from the temporal margin of the eyelids; the other was placed about 10 mm below the margin of the lower eyelid. A square electrode (32 × 32 mm) was positioned on the forehead and served as ground electrode.

The OO-EMG was measured simultaneously with lid movement. In a few experiments, the electrical stimulus delivered on the side where the coil was attached exhibited an interference with the lid movement recording. To record the R_1 simultaneously with lid movement properly, EMG recording was suppressed 4 ms after stimulus onset by the computer. The investigator determined the onset and the offset of OO-EMG activity for each trial. The computer calculated the integrated OO-EMG by adding all OO-EMG activity between start and end of the rectified EMG activity.

Experimental procedures

Two measurement methods were used: 1) the fixation point method for blink recordings, and 2) the eye-dependent positioning method. The subject was positioned comfortably on a chair, the subject's head was stabilized on a head holder, and his/her chin and forehead were fixed at a standard position. The measured eyelid was placed in the center of the magnetic field. The subject faced a black 2 × 2 m flat screen from a distance of 160 cm with 29 light-emitting diodes (LED). All LEDs except one, the fixation point, were positioned in three concentric circles: 4 LEDs were positioned at 0°, 90°, 180°, and 270° at a 10° radius; 12 LEDs were positioned at 0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270°, 300°, and 330° at 20° and 30° radii; and the 29th LED (position 0–0) was positioned in the center of these circles and exactly in front of the measured eye.

SPONTANEOUS BLINKS. To record spontaneous blinks, subjects were asked to watch a video on a 21 × 28-cm television screen from a distance of 160 cm. To avoid subject's attention from the experimental setting, all blinks were registered between 10 and 25 min after the start of a movie on the screen.

FIG. 1. Schematic representation of kinematics of an upper eyelid movement and the simultaneously recorded orbicularis oculi EMG (OO-EMG) of a normal adult with trigeminal reflex blinking after corneal air puff stimulation. Relationship between lid saccade amplitude and the up phase duration is indicated. Tmax, time of maximal down phase amplitude reached; T-up-phase, the up phase time duration is defined as the termination of the blink minus the time the maximal deviation. Integrated OO-EMG (black area) is measured in microvolts times seconds ($\mu V \cdot s$).

VOLUNTARY BLINKS. To obtain voluntary blinks, two types of experiments were performed. In the first experiment, the subject was asked to fixate the center LED (position 0–0) and blink as fast as he could every time after the LED flashed. An experiment consisted of 20 trials with an interval of 6 s. In the second experiment, the subject was asked to fixate the center LED until it was inactivated. Subsequently, a randomly selected LED in the field was lit, and the subject was asked to make a saccade towards this LED and to blink directly after reaching the target. After the blink, the subject was asked to return immediately to the zero position and gaze to the center LED waiting for the next trial. This experiment continued until all 28 LED positions had been presented. The same procedure was followed for reflex blinks, except that a stimulus was offered when subject's eye reached the desired LED position followed by a reflex blink.

AIR PUFF STIMULATION. The corneal reflex was evoked by an air puff delivered to the cornea ipsi- or contralaterally to the recorded eyelid. This was done to measure small latency differences between the stimulated and nonstimulated sides. Air puff stimuli were generated by a pressure unit, which was connected by a rigid plastic tube to a small buffer reservoir. Specific air puff intensity was guaranteed by this reservoir.

The sound click produced by the air valve and regulated by the computer never elicited an acoustic reflex blink. The air pulses had a duration of 11 ms at an intensity of 2 Bar and 13 ms at intensities of 4 and 6 Bar. Air puffs were fed through a 4.0-mm-diam plastic tube with a 2.0-mm-diam tip, positioned at 10 mm from the cornea in a fronto-lateral eye position. The fixed delay of the stimulus at the cornea was 15 ms after the computer opened up the small cylinder, which was measured with a microphone. All data were corrected for this delay.

SO NERVE STIMULATION. The blink reflex was evoked by an electrical stimulus to the SO nerve. For this purpose, the cathode was placed on the supraorbital foramen and the anode was positioned 2 cm laterally on the forehead. Resistance of the electrodes was always 5 kΩ or lower. Blinks were recorded after supramaximal stimulation, which was defined for every individual as three times the threshold value.

Both the electrical and air puff methods for eliciting reflex blinks followed the same procedures. First the subject looked straight ahead, and in two successive trials, electrical and air puff, the stimuli were delivered 20 times with 6-s intervals. Second, the 28 LEDs lit up randomly and the stimuli were delivered after the subject's gaze was directed towards the LED.

Calibration of the search coil

To calibrate the search coils, a device was developed adapted from the one described by Evinger et al. (1991). This device could rotate the coil in the vertical and horizontal direction at the same point in the magnetic field where the subject's eyelid is positioned during recording of the blink. Using these coil rotations, it was possible to record a change in the magnetic field for every 5° between −45° and +45°. A linear correlation was found between −30° and +30°. Simultaneous video recordings during different types of blinks confirmed this. In this way, all changes of the vertical component, observed in the magnetic field, were proportional to the eyelid rotation, and the vertical component could by expressed as an angle. The line measured starts to deviate little from a straight line at −35° and +35° and tends towards a sinus form where angles of −45° and +45° are recorded. In the range between −35° and −45° and +35° and +45°, a deviation from linearity of 3.9° (at −45°; 41.1 ± 0.2°) was measured. At 90° a sinus form was reached, when changes in magnetic field were plotted against the rotation angle of the coil.

Data acquisition

Timing of the stimulus events was done using an Asus Pentium-controlled data acquisition III PC, equipped with a data-acquisition board (DAS 1800 HC Series, Keithley Instruments). Horizontal and vertical eyelid position signals were amplified, low-pass filtered (318 Hz), and sampled with 12-bit resolution/ms. The raw data were stored on disk for off-line analysis.

The OO-EMG was recorded by a Medelec amplifier: 32–800 Hz (6 dB/octave), filtered with a 50-Hz notch filter, and digitally filtered between 100 and 300 Hz (6 dB/octave). The EMG recordings were rectified. For each trial, the computer displayed OO-EMG and lid movement in vertical and horizontal directions.

RESULTS

In all subjects, gazing at the fixation point of the flat screen, simultaneous OO-EMG activity, and eyelid movements were recorded during spontaneous and voluntary blinks, and after air puff and SO stimulation. When the down phase amplitude was plotted against the integrated OO-EMG, two of the five subjects showed a relative high correlation in these types of blinking (Fig. 2). Four subjects successfully achieved the 28 randomly offered LED positions at the flat screen during voluntary blinking and after air puff and SO nerve stimulation.
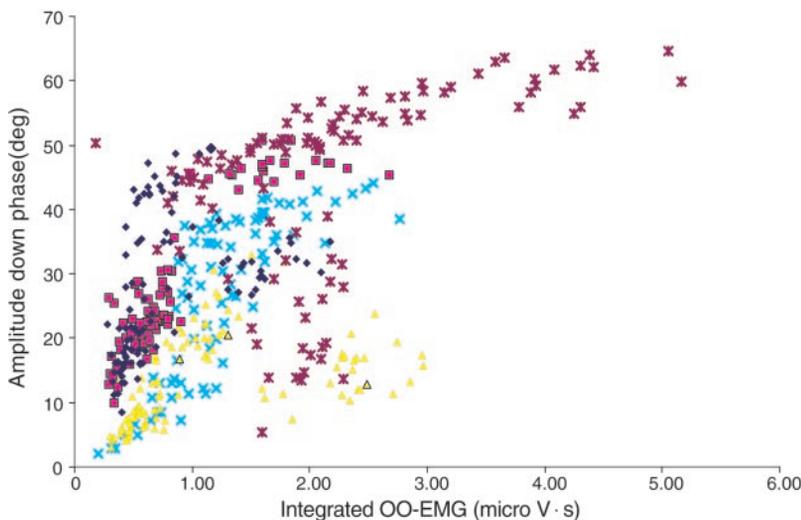
FIG. 2. Blink kinematics for the down phase of all types of blinks of 5 normal adults. The maximal down phase amplitude is plotted against the integrated OO-EMG. Each subject is presented by a specific mark. Data pools of subjects A and B show a relatively high correlation, $R^2 = 0.7952$ and $R^2 = 0.6122$, respectively. Subjects: A, X; B, △; C, ■; D, *; E, ◆.

Simultaneously eyelid positions and OO-EMG activity were measured. These recordings demonstrated during voluntary blinks a gradual increase of EMG activity from the $+30°$ upward towards the $-30°$ downward position and a gradual decrease of the maximal down phase amplitude from the $+30°$ upward towards the $-30°$ downward position. In general, 5–6 ms after the OO-EMG onset, rapid lowering of the upper eyelid started until the OO-EMG ceased, after which the eyelid returned more slowly towards its starting position.

Fixation point and blinking

The total duration of spontaneous blinks (Fig. 3*A*) was 334 ± 67 ms, the down phase duration was 92 ± 17 ms, and the up phase duration lasted 242 ± 55 ms, which is almost three times longer than the down phase. The maximal down phase amplitude was $21.3 \pm 5.9°$, and the maximal downward velocity was $565 \pm 297°/s$. The simultaneously measured integrated OO-EMG was 4.0 ± 0.9 $\mu V \cdot s$.

Voluntary blinks (Fig. 3*B*) had a shorter total duration of 275 ± 37 ms and were less variable in duration compared with the spontaneous blink. The down phase duration was 88 ± 13 ms and the up phase duration was 187 ± 34 ms. The maximal down phase amplitude was $38 \pm 10°$, the maximal downward velocity was $1402 \pm 159°/s$, and the concomitantly measured integrated OO-EMG was 3.2 ± 0.9 $\mu V \cdot s$.

The electrically stimulated (SO) blink reflex had a total duration of 205 ± 18 ms, the down phase duration was 53 ± 6 ms, and the up phase duration lasted 152 ± 6 ms (Fig. 3*C*). The lid movement started at 38 ± 3 ms. The maximal down phase amplitude was $31 \pm 18°$ and the maximal downward velocity was $2302 \pm 65°/s$. The simultaneously registered integrated OO-EMG of the R_2 was 6.1 ± 1.5 $\mu V \cdot s$.

The ipsilateral air puff–evoked blink reflex had a total duration of 304 ± 34 ms, the down phase duration was 87 ± 15 ms, and the up phase duration was 217 ± 32 ms (Fig. 4*A*). The lid movement started at 33 ± 7 ms. The maximal down phase amplitude was $28 \pm 19°$, and the maximal downward velocity was 2497 ± 208 °/s. The simultaneously measured integrated OO-EMG was 9.7 ± 3.1 $\mu V \cdot s$.

The contralateral air puff–evoked blink reflex had a shorter total duration of 244 ± 22 ms, the down phase duration was 70 ± 11 ms, and the up phase duration was 173 ± 14 ms. (Fig. 4*B*). The lid movement started at 33 ± 6 ms. The maximal down phase amplitude was $25 \pm 19°$, and the maximal downward velocity was $2146 \pm 160°/s$. The simultaneously measured integrated OO-EMG was 8.0 ± 1.8 $\mu V \cdot s$. Superposition of 15 successive trials of ipsi- as well as contralateral air puff–evoked blink reflexes (Fig. 4, *C* and *D*) showed a stereotype profile of the down and up phase with minimal variability and without blink oscillations.

For a single subject, parameters of five different types of blinking were plotted against the maximal down phase amplitude. When the maximal downward velocity was plotted
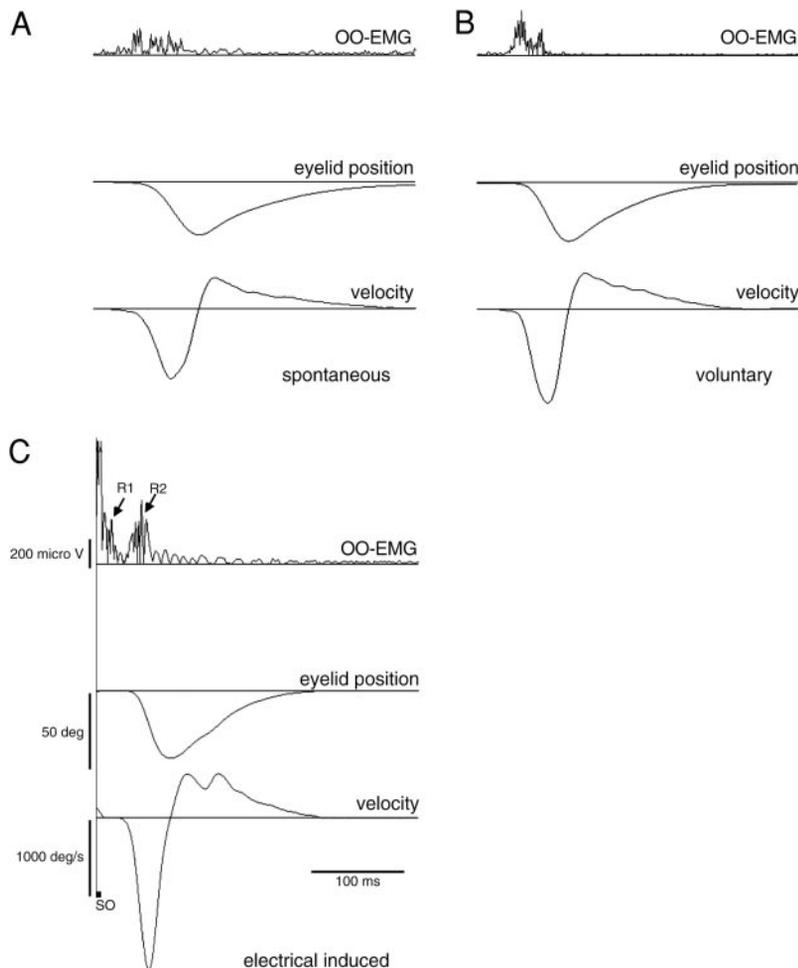
FIG. 3. Kinematics of upper eyelid movements and simultaneously recorded OO-EMG activity with blinking. *A*: spontaneous blinking, records of OO-EMG, upper eyelid position, and maximal downward velocity. *B*: voluntary blinking, records of OO-EMG, upper eyelid position, and maximal downward velocity. *C*: trigeminal reflex blinking following electrical stimulation of the supraorbital nerve ipsilateral to the recorded right side, records of OO-EMG (R_1 and R_2 responses are indicated), upper eyelid position, and maximal downward velocity.
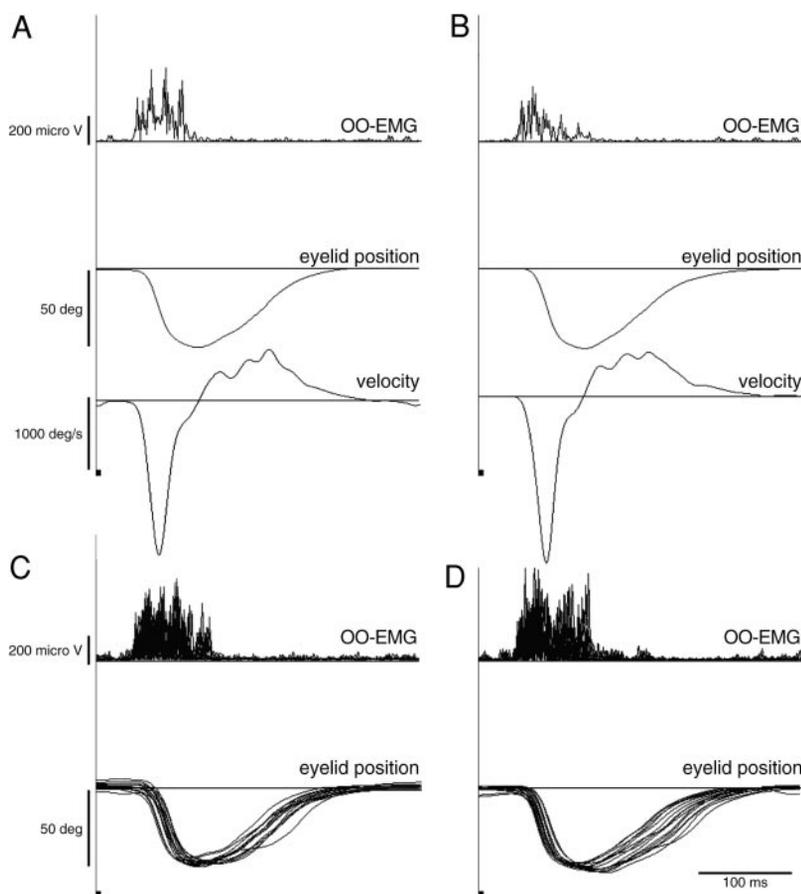
FIG. 4. Kinematics of upper eyelid movements and simultaneously recorded OO-EMG activity with blinking. *A*: trigeminal reflex blinking following corneal stimulation by an air puff (4 ms, 2 Bar) ipsilateral to the recorded right side, records of OO-EMG, upper eyelid position, and maximal downward velocity. *B*: trigeminal reflex blinking following corneal stimulation by an air puff (4 ms, 2 Bar) contralateral to the recorded right side, records of OO-EMG, upper eyelid position, and maximal downward velocity. *C*: superposition of 15 successive traces of ipsilateral corneal air puff induced blinking and their OO-EMG activities. *D*: superposition of 15 successive traces of contralateral corneal air puff–induced blinking and their OO-EMG activities.

against the maximal down phase amplitude, a significant correlation was found for spontaneous blinks r (correlation coefficient) $= 0.665$ ($P < 0.001$) and for electrical induced blinks $r = 0.821$ ($P < 0.001$). For voluntary blinks, $r = 0.266$ (not significant); for ipsilateral air puff induced blinks, $r = 0.258$ (not significant); and for contralateral air puff–induced blinks, $r = 0.225$ (not significant) (Fig. 5, *A–C*). When the down phase duration was plotted against the maximal down phase amplitude, a correlation was found for spontaneous blinks, $r = 0.442$ ($P = 0.05$); for ipsilateral air puff–induced blinks, $r = 0.482$ ($P < 0.05$); for contralateral air puff–induced blinks, $r = 0.627$ ($P < 0.01$); for electrically induced blinks, $r = 0.550$ ($P < 0.02$); and for voluntary blinks, $r = 0.056$ (not significant; Fig. 5, *D–F*). When blink duration was plotted against the maximal down phase amplitude, a correlation was found for spontaneous blinks $r = 0.560$ ($P < 0.01$) and for contralateral air puff–induced blinks $r = 0.508$ ($P < 0.05$). For voluntary blinks, $r = 0.413$; for ipsilateral air puff–induced blinks, $r = 0.003$ (not significant); and for electrically induced blinks, $r = 0.382$ (not significant) (Fig. 5, *G–I*). When the peak time was plotted against the maximal down phase amplitude, a correlation was found for ipsi- and contralateral air puff–induced blinks, $r = 0.453$ ($P = 0.05$) and $r = 0.775$ ($P < 0.001$), respectively; for electrically induced blinks, $r = 0.317$; these blinks were not significant (Fig. 5*J*). A correlation was also present when OO-EMG was plotted against the maximal down phase amplitude. For spontaneous blinks, $r = 0.552$ ($P < 0.01$); for ipsilateral air puff–induced blinks, $r = 0.843$ ($P < 0.001$); and for contralateral air puff–induced blinks, $r = 0.580$ ($P < 0.02$). For voluntary blinks, $r = 0.253$ (not significant);

for electrically induced blinks, $r = 0.211$ (not significant) (Fig. 5, *K–M*).

Electromyography of the OO muscle

A marked variability in integrated OO-EMG activity can be observed in the different types of blinking. The onset of the lid movement and the simultaneously recorded OO muscle activity was analyzed for the voluntary, electrically, and the ipsilateral and contralateral air puff–induced blinks. The integrated OO-EMG for voluntary blinking was 3.2 ± 0.9 $\mu V \cdot s$. From the ipsilateral SO nerve-evoked blink, the R_1 component of the OO-EMG started at 13 ± 1 ms, the R_2 component was registered at 32 ± 3 ms, and the onset of the lid movement was 38 ± 3 ms. The latency difference between the OO muscle activity and the lid movement was 6 ms (Table 1).

The OO-EMG activity of the ipsilateral air puff–induced blink started at 24 ± 5 ms, and the integrated OO-EMG was 9.7 ± 3.1 $\mu V \cdot s$. The onset of lid movement of ipsilateral air puff–induced blinks was 33 ± 7 ms. The latency difference between the OO muscle activity and the lid movement was 9 ms (Table 1).

The OO-EMG activity of the contralateral air puff–induced blink started at 28 ± 3 ms, and the integrated OO-EMG was 8.0 ± 1.8 $\mu V \cdot s$. The onset of lid movement of contralateral air puff–induced blinks was 33 ± 6 ms. The latency difference between the OO muscle activity and the lid movement was 5 ms (Table 1).

When the down amplitude phase was plotted against the integrated OO-EMG of all recorded blinks (Fig. 2), the mean
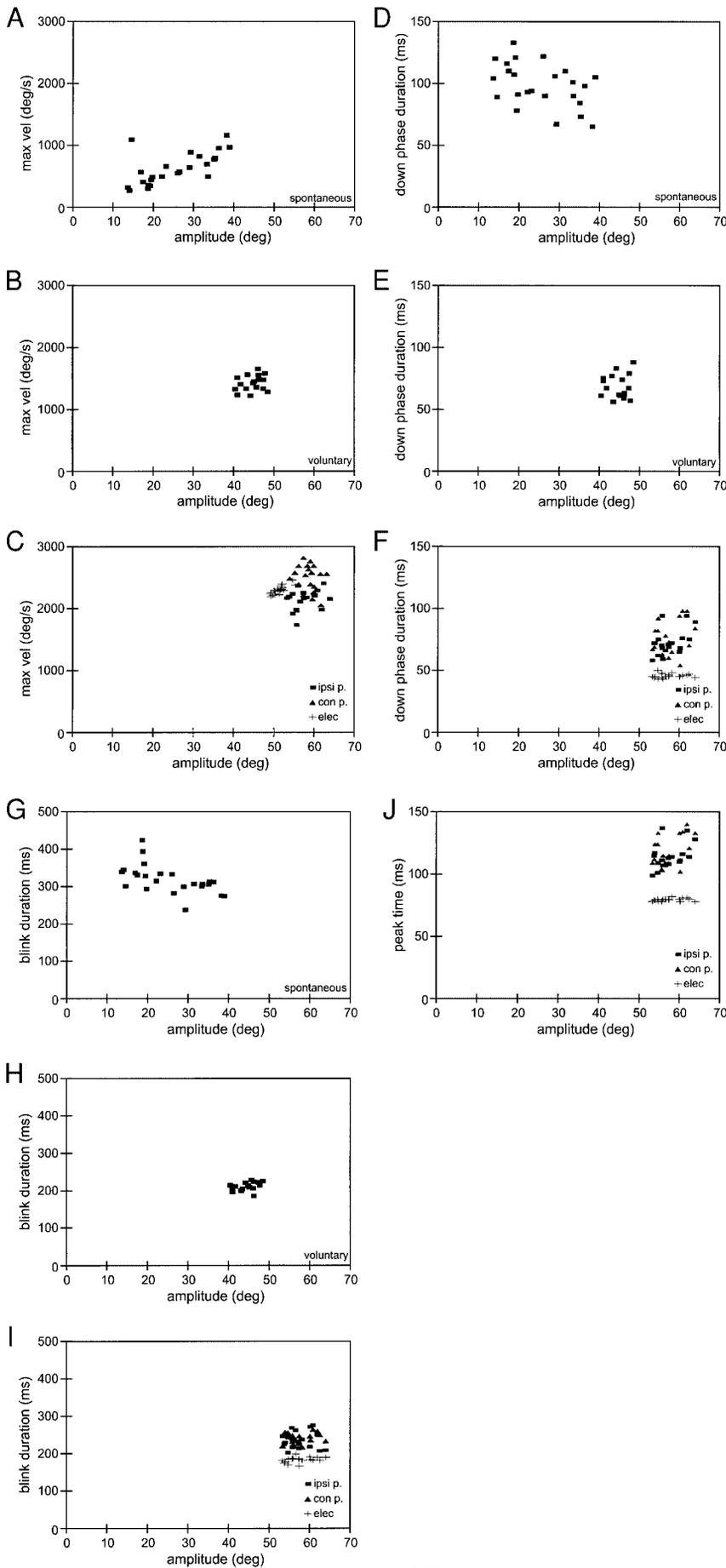
FIG. 5. Lid movement kinematics of spontaneous, voluntary, and reflex blinking. Maximal downward velocity (max vel) plotted against the maximal down phase amplitude from the same subject (*A–C*). Down phase duration plotted against the maximal down phase amplitude (*D–F*). Blink duration plotted against the maximal down phase amplitude (*G–I*). Peak time plotted against the maximal down phase amplitude (*J*). OO-EMG plotted against the maximal down phase amplitude (*K–M*). Each point represents an individual lid movement; correlation is indicated in the text. Spontaneous blinks are the lowest in maximal downward velocity. Electrical induced blinks are the most stable in maximal downward velocity and down phase duration. ipsi p., ipsilateral air puff induced blink; con p., contralateral air puff induced blink; elec, electrical induced blink.
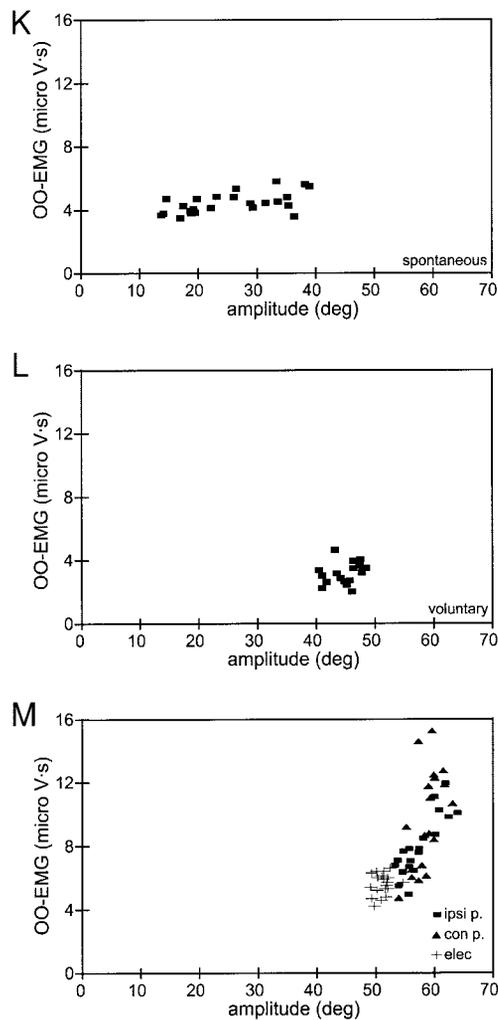
FIG. 5. (*continued*)

correlation coefficient was 0.61 ($y = 0.106x + 14.58$). Per subject, the correlation coefficients varied: 0.42, 0.43, 0.47, 0.78, and 0.89. The youngest subject (22 yr old) shows the highest correlation.

Effect of the eye position on blinking

Voluntary and reflex blinks were recorded in four subjects. The subjects gazed towards the 28 randomly presented LED positions. Blinks could be compared in their maximal down phase amplitude, maximal downward velocity, and integrated OO-EMG at different eye positions (Fig. 6, *A–C*).

When the starting position (angle) is plotted against maximal down phase amplitude (Fig. 6*A*) or maximal downward velocity (Fig. 6*B*), an almost linear relation can be observed in voluntary, air puff, and electrically induced blinking. When maximal down phase amplitude is plotted against eye position, for voluntary blinks, $r = 0.994$ ($P < 0.001$); for air puff–induced blinks, $r = 0.9997$ ($P < 0.001$); and for electrically induced blinks, $r = 0.886$ ($P < 0.02$). When maximal downward velocity is plotted against position, for voluntary blinks, $r = 0.987$ ($P < 0.001$); for air puff–induced blinks, $r = 0.913$ ($P = 0.01$); and for electrically induced blinks, $r = 0.963$ ($P < 0.01$). When integrated OO-EMG is plotted against position, for voluntary blinks, $r = 0.980$ ($P < 0.001$); for air puff–induced blinks, $r = 0.438$ (not significant); and for electrically induced blinks, $r = 0.665$ (not significant).

In a single subject, the total blink duration in the upward gaze position of voluntary blinking is relatively short, and the maximal downward velocity is relatively high (Fig. 7, *A* and *B*). The maximal downward velocities recorded in four subjects at upward gaze position of 30° ranged between 493 and 2166°/s, and at 30° downward position, it ranged between 227 and 1100°/s. The maximal down phase amplitude at 30° upward position ranged between 17° and 55.7°, and at 30° downward position, it ranged between 9.6° and 29.8°. The total blink duration at 30° upward gaze was between 121 and 163 ms, while at the 30° downward position, it ranged between 154 and 203 ms. In the upward gaze position, the integrated OO-EMG activity was relatively low and varied between 2.6 and 4.8 μV · s at the 30° upward gaze position. At the 30° downward gaze position, it ranged between 4.5 and 6.3 μV · s (Fig. 7*C*). These

TABLE 1. *Comparison of results of the current study with other studies*

Type of Blinking	Author	Comments	Down Phase	Up Phase	Amplitude	Onset Movement	Onset R1	Onset R2	Delay
Spontaneous	Current study		92 ± 17	242 ± 55	21.3 ± 5.9°				
	Evinger et al. (1991)		Slowest	Slowest	Max 6 mm				10.8 ± 4.1
	Guitton et al. (1991)		85	200	41.7°				
	Sun et al. (1997)		84.5	198.2	33.2°				
	Stava et al. (1994)	Left	104.7 ± 28						
	Stava et al. (1994)	Right	98.0 ± 26						
Voluntary	Current study		88 ± 13	187 ± 34	38.5 ± 9.9°				11 ± 4
	Evinger et al. (1991)		Middle	Similar to SO					
	Collewijn et al. (1985)		100–150	300	7.5°				
	Sun et al. (1997)		91.86	221.1	44.82 ± 5°				
SO stimulated	Current study		53 ± 6	152 ± 6	31.0 ± 18.0°	38 ± 3	13 ± 1	32 ± 3	6
	Snow and Frith (1989)	Early *R1* movement					10.3 ± 0.8	32 ± 2.4	
	Evinger et al. (1991)	Early *R1* movement	Fastest	Similar to voluntary		24.1 ± 6.5	12.2 ± 7.8	41.7 ± 7.8	11.9
	Evinger et al. (1991)					55.1 ± 11.4		41.7 ± 7.8	13.4
	Bour et al. (2000)					49.4 ± 5.8	10–12	30.4 ± 2.3	19.0
	Bour et al. (2000)	Early *R1* movement				15		30.4 ± 2.3	5
Air puff–induced ipsilateral	Current study		87 ± 15	216 ± 32	28.0 ± 18.0°	33 ± 7		24 ± 5	9
Air puff–induced contralateral	Current study		70 ± 11	173 ± 14	24.7 ± 19.0°	33 ± 6		28 ± 3	5

Values are means with SD in milliseconds, except for the amplitude, which is shown in degrees.
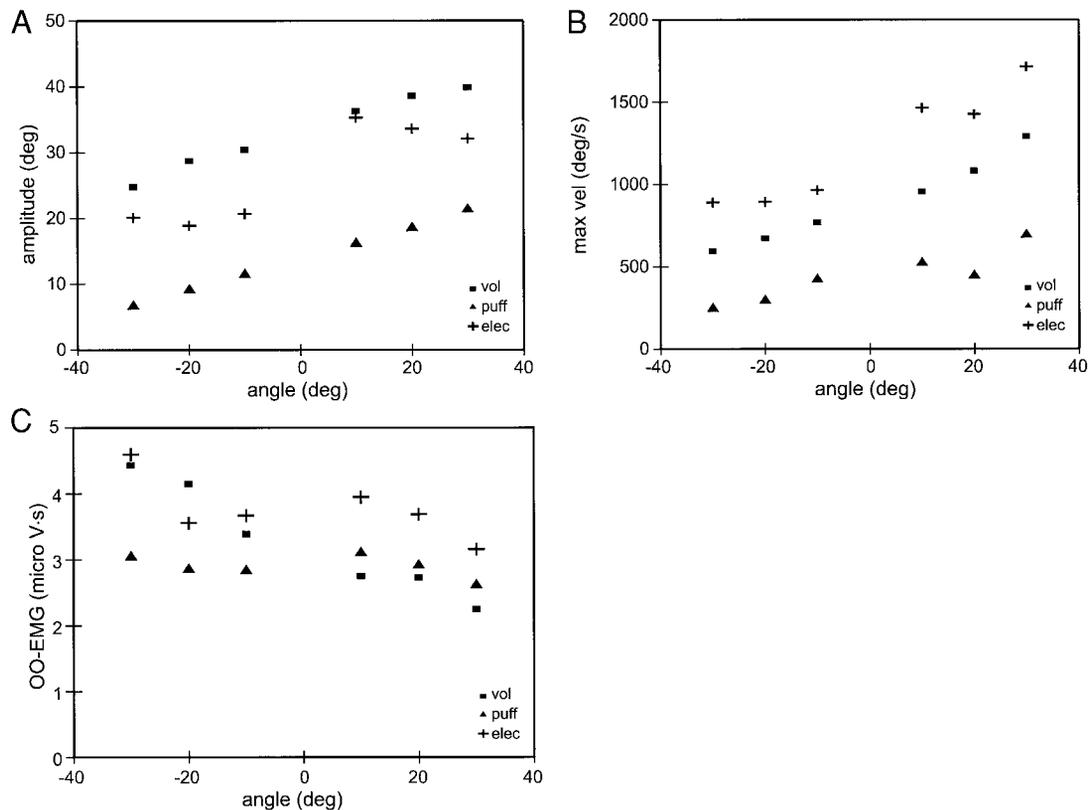
FIG. 6. Average of maximal down phase amplitude (*A*), maximal downward velocity (*B*), and integrated O-EMG (*C*) lid movement kinematics from 4 subjects plotted against the initial eye position (angle). Correlation coefficient for maximal down phase amplitude, maximal downward velocity, and integrated OO-EMG in voluntary blinking is 0.994, 0.987, and 0.980, respectively. In voluntary blinks, an increasing gradient is present in maximal down phase amplitude and maximal downward velocity from −30° to +30°, and a decreasing gradient is present in integrated OO-EMG from −30° to +30°. vol, voluntary blinks; puff, air puff induced blinks; elec, electrical induced blinks.

upward and downward gradients were more pronounced in electrically (Fig. 8, *A* and *B*) and air puff–induced reflex blinks (Fig. 9, *A* and *B*). Figure 8*A* shows the simultaneously measured integrated OO-EMG activity during electrically induced reflex blinks. The maximal down phase amplitude at 30° upward gaze position was 45.4° and the concomitantly integrated OO-EMG was 4.6 μV · s. The maximal down phase amplitude at 30° downward position was 34.9° and the concomitantly integrated OO-EMG activity was 6.1 μV · s. Gaze shifts to the right or the left did not show appreciable differences in maximal down phase amplitude and total blink duration.

In the same subject, the maximal downward velocity at 30° upward gaze position was 2470°/s, and at the 30° downward gaze position, it was 1605°/s. The total blink duration was 127 ms at 30° upward gaze position and 181 ms at 30° downward gaze position.

DISCUSSION

As Evinger et al. (1991) have shown, the motion of the eyelids during a blink is a combination of rotation and translation. In this study, we used the magnetic search coil technique (Remmel 1984) for measuring the lid rotation. To calibrate the coil displacement in the magnetic field, most investigators used the method of Becker and Fuchs (1988); sometimes this method was modified (Collewijn et al. 1985). In the Becker and Fuchs method, a lever is placed perpendicular

on the coil, and by the use of a protractor, placed parallel to the lateral part of the face; the angle of rotation is read from the lever. The accuracy of this method strongly depends on the observer. Gaze positions are limited to more than +20° and –20°. In the current study, these limits were avoided, and gaze positions up to −35° and +35° could be calibrated with the developed device. Calibration recordings showed that small deviations occurred at –35° and +35°, which lay outside the range of actual angles recorded during experiments.

Electrically induced blinks present the most stable pattern in lid movement and integrated OO-EMG and revealed the shortest total blink duration, the shortest down and up phases, and the fastest maximal downward velocity (Table 1).

Many authors (Becker and Fuchs 1988; Evinger et al. 1984, 1991; Gittins et al. 1999; Hasan et al. 1997; Niida et al. 1987; Sun et al. 1997) showed a linear relationship between maximum downward velocity and maximal down phase amplitude. In this study, this relationship was confirmed.

With respect to the up phase of a lid movement, an objective method has not been reported to determine the end of a blink. Because the eyelid does not always return to its original position and the end position is not always stable, it has been difficult to determine the end of each blink accurately. Several authors described the lid duration by defining general variables in a line diagram (Evinger et al. 1991; Peshori et al. 2001), indicating the offset of the up phase of lid movement only marginally (Evinger et al. 1991). In the current study, an
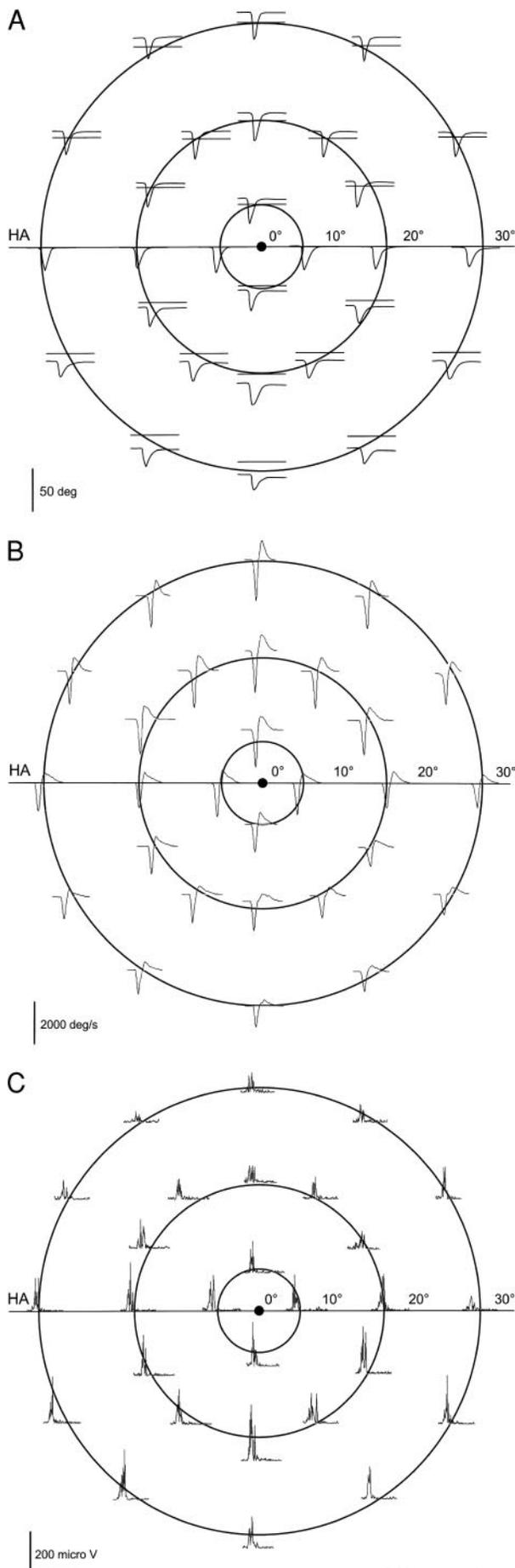
objective variable has been introduced by defining the duration of the upward phase as 95% of the maximal swerve of the down phase.

Fixation point and blinking

The profiles of spontaneous blinks and the duration of down and up phases measured in the current study were in agreement with results in other studies, but the maximal down phase amplitudes differed significantly (Stava et al. 1994; Sun et al. 1997). In this study, a wide variability of total spontaneous blink duration was recorded, which ranged from a gently short blink, accompanied with a specific small integrated OO-EMG, to a more forceful blink, accompanied with a large integrated OO-EMG. Evinger et al. (1991) found an average down phase duration of 75 ms, while the up phase duration ranged between 140 and 225 ms. In comparison with the study of Sun et al. (1997), the maximal down phase amplitude registered in the current study was relatively small (21.3 ± 5.9°). As the down and up phase duration were rather similar in the preceding mentioned studies, maximal down phase amplitude and maximal downward velocities recorded in the current study were significantly different from their observations.

The profiles of voluntary blinks, e.g., maximal down phase amplitude and down phase and up phase duration, recorded in this study were similar to those of other studies (Evinger et al. 1991; Niida et al. 1987). Noteworthy are the observations that voluntary integrated OO-EMG is greater than spontaneous integrated OO-EMG, that down and up phase duration is shorter, and that maximal down phase amplitude and maximal downward velocity are greater than that with spontaneous blinks. This indicates that relatively more fast OO motoneurones are recruited in voluntary blinks and therefore more LPS motoneurons should be involved for the up phase during voluntary blinking. Our data did not agree with that of Collewijn et al. (1985), who used a calibration method developed from that of Evinger et al. (1984). These authors attached the search coil anterior to a light wooden lever and posterior to the eyelid and registered vertical and horizontal rotations of this coil. They measured a longer down phase duration and an almost three times longer up phase duration of 300 ms, accompanied by five times lower maximal downward amplitudes of 7.5°, which is unrealistic. Moreover, they recorded a substantial horizontal displacement of the eyelid, which was one-half the value of a vertical displacement. Unfortunately, no metrics of eyelid movements were reported in their paper, which reduces the value of their observations. In the current study, we could hardly find any horizontal displacement in accordance with simultaneous video recordings of eyelid movements.

Few data are available regarding ipsilateral and contralateral air puff–induced blinks in humans. Evinger et al. (1984) stud-

FIG. 7. Chart of upper eyelid movements and simultaneous OO-EMG, demonstrating the kinematics at 28 different starting eye positions of voluntary blinking. *A*: upper eyelid positions, in starting eye positions above the horizontal axis (HA), blink duration is the shortest, and maximal down phase amplitudes are largest. In starting eye positions below HA, blink duration is longest, and maximal down phase amplitudes are smallest. *B*: upper lid velocities, maximum down- and upward velocities are largest above HA compared with lid velocities below HA. *C*: records of integrated OO-EMG; in contrast to maximal down phase amplitudes of upper eyelid positions and maximal downward velocities, OO-EMGs are remarkably smaller in starting eye positions above HA.

A

50 deg

B

2000 deg/s

C

200 micro V
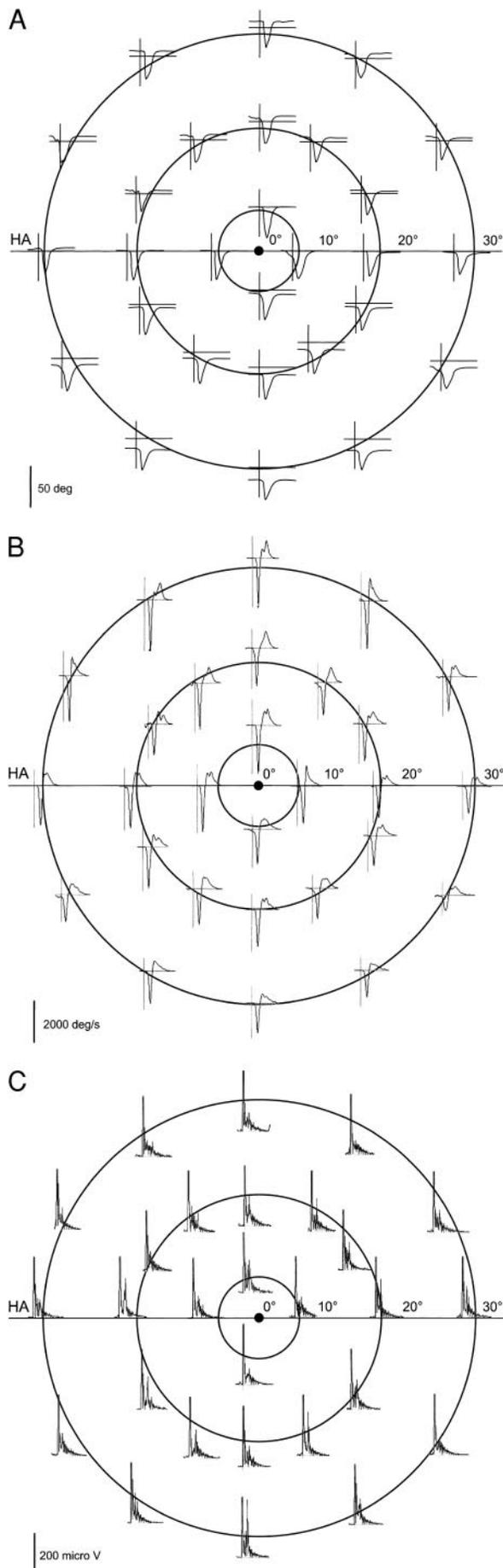
ied reflex blinks in humans after a gentle air puff to the periorbital region. The down phase duration was 87 ± 47 ms, which is comparable with our findings. The current study showed a fast down phase bilaterally, followed ipsilaterally by a small delayed up phase of 4 ms. The total duration varied between 240 and 300 ms. The lid kinematics and simultaneous OO-EMG showed a remarkable longer duration of the ipsilaterally induced blink in the up phase. This difference may be due to neuronal factors, where the sensory trigeminal complex might effect the premotor area of levator palpebrae motoneurons, which are in turn responsible for lid elevation (Horn et al. 2000; Ndiaye et al. 2002).

The duration of electrically induced blinks was shorter and more stable than that of voluntary, spontaneous, and air puff–induced blinks, and their profiles were in agreement with other studies (Bour et al. 2000; Evinger et al. 1991; Snow and Frith 1989). In the study of Bour et al. (2000), the onset of lid movement was significantly earlier than the onset of the late R_2 response in this study. The early lid component was not always registered and strongly depended on the subject, which was in agreement with observations of Hammond et al. (1996), where in 24% of the trials, an early shallow lid closure was found.

In several studies, the onset of lid movement and OO muscle activity were registered in electrically induced blink reflex (Bour et al. 2000; Evinger et al. 1991; Peshori et al. 2001; Snow and Frith 1989), using different methods of recording to those used in the current study. Evinger et al. (1991) reported an earlier onset of lid movement than was found in the current study. This could be attributable to the age of the subjects. The extensive study of Peshori et al. (2001) showed in normal humans over 60 yr of age that the mean lid-closing duration and the excitability and latency of the electrically induced blink increased significantly compared with younger subjects. This was also observed in the current study, where the youngest subject showed the shortest total blink duration and the highest maximal down phase amplitude. These authors suggested an age-related loss of dopamine neurons as the cause of longer blink times.

Electromyography of the OO muscle

The current study showed a significant correlation between integrated OO-EMG and maximal down phase amplitude for all blinking types. The most obvious correlation's were seen in small fast spontaneous and electrically induced blinks.

During spontaneous blinking, OO-EMG-activity was measured during the initial phase in the pretarsal portion and not in the preseptal and orbital portions (Niida et al. 1987). These authors postulated that the pretarsal portion of the OO muscle

FIG. 8. Chart of upper eyelid movements and simultaneous OO-EMG, demonstrating the kinematics at 28 different starting eye positions of electrical evoked trigeminal blinking. *A*: upper eyelid positions, in starting eye positions above the HA and in starting eye positions 20–240, 20–270, 20–300, and 20–330, blink duration is shortest and the maximal down phase amplitudes largest. *B*: upper lid velocities, maximum down- and upward velocities are largest in starting eye positions above HA and in starting eye positions 20–240, 20–270, 20–300, and 20–330. *C*: records of OO-EMG; in contrast to maximal down phase amplitudes of upper eyelid positions and maximal downward velocities, OO-EMGs are smaller above HA and in starting eye positions 20–240, 20–270, 20–300, and 20–330 compared with starting eye positions 30–240, 30–270, 30–300, and 30–330. In all profiles, a stimulus artifact is present.
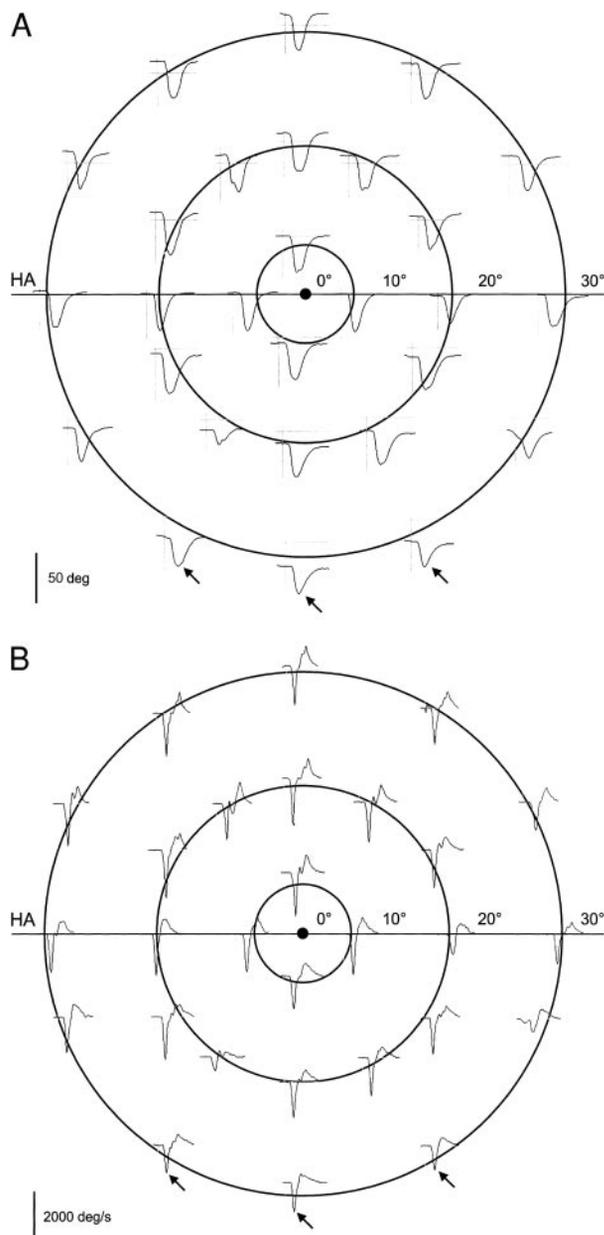
FIG. 9. Chart of upper eyelid movements demonstrating the kinematics at 28 different eye positions of corneal air puff induced blinking (4 ms, 2 Bar). Positions 20–240 and 30–330 deviate from nearby recordings, probably due to artifacts. *A*: from all eye positions, blink duration are comparable. Maximal down phase amplitudes differ below the HA. Positions 30–240, 30–270, and 30–300 (arrowheads) show lower amplitudes compared with the remaining positions. *B*: upper lid velocities. The maximum down- and up phase velocities above HA are larger compared with maximal velocities of positions 30–240, 30–270, and 30–300.

was exclusively reserved for fast synchronous spontaneous blinking. This observation of subdivision activities is in line with findings in neuronal tracing studies in the monkey, where in the intermediate subnucleus, an exclusive dome-like portion of facial motoneurons is located subserving the pretarsal OO muscle portion (VanderWerf et al. 1998).

Evinger et al. compared lid kinematics with rectified OO-EMG and found, like many other authors (Bour et al. 2000; Hammond et al. 1996; Snow and Frith 1989), a concomitant initial lid movement and R_1 response of electrically induced

blinks. In these studies, the onset of the R_1 and the R_2 response agree very well, although Evinger et al. (1991) found an R_2 onset with a longer latency. Because probably not all subjects received stimuli with the same maximal intensity in every trial, we observed, in agreement with Evinger et al. (1991), differences in onset at stimulation levels of two to three times the threshold.

Effect of eye position on the eyelid movement

Three main observations may be made from the eye position–dependent experiments.

The first two observations were that in voluntary and reflex blinking, the largest maximal down phase amplitude and maximal downward velocity were recorded as the gaze is directed in the 30° upward position. This is in agreement with observations in human (Bour et al. 2000; Collewijn et al. 1985) and in cats (Gruart et al. 1995), where the initial down phase of air puff–induced blinks increased in maximal down phase amplitude and decreased in latency.

The third observation was that, in voluntary blinking, OO-EMG increased from the upward to the downward positions. In voluntary and reflex blinking, the highest values are recorded in the 30° downward position. Apparently, during reflex blinking, a relative constant subset of motoneurons is activated in all eye positions except the lowest one, while in voluntary blinking, a variable subset of motoneurons is activated depending to the eye position. This indicates that different subsets of facial motoneurons are recruited for voluntary and reflex blinking.

Another unexpected finding was the relatively high maximal down phase amplitudes recorded in the most downward eye position in voluntary and reflex blinks. During an upward gaze, the OO muscle is silent, and a greater distance has to be covered by the upper eyelid before it reaches the lower eyelid than in a downward gaze. When the gaze is directed downward, only a few millimeters of displacement is required before the lower eyelid is reached. In contrast to the up gaze position, the blink's duration seems to be longer as the gaze is directed downward. A possible explanation might be that at gaze position –30°, the lid position is out of the range of normal physiological conditions, since in a free head position an associated compensatory head movement can mostly be present from gaze position −20° (Herst et al. 2001). Another explanation might be that lowering of the upper eyelid to its starting position and maintaining this position in the gaze shift slightly increases the baseline OO muscle activity.

There was no relation between the maximal down phase amplitude and blink duration when the subject was looking straight ahead. In the current study, no differences could be found between gaze shifts to the left or right, which is supported by findings in cats (Gruart et al. 1995).

Functional considerations

The data presented in this study show important additional information about eyelid kinematics under various conditions. In an earlier eye position study, a correlation was found between the extent of the movement of both eyes and the initial gaze position (Bour et al. 2000). For, at least, voluntary blinking, our experiments show that the initial eye position also defines the extent of eyelid displacement, their associated total

duration, maximal down phase amplitude, maximal downward velocity, and integrated OO-EMG. For reflex blinking, only the lowest eye position influenced vertical lid displacement. These observations indicate that, for voluntary blinking, the neuronal circuit of eyelid movements (partly) shares or is controlled by the neuronal blinking circuit, but for reflex blinking, the neuronal circuit of eyelid movements is more or less operating independently from the blinking circuit.

In all types of blinks, the down phase is more or less constant, while the up phase varies in duration, maximal amplitude, and maximal velocity. In contrast with electrically induced blinks, air puff–induced blinks lack an ipsilateral R_1 component. These findings indicate that afferent fibers in the trigeminal nerve responsible for the R_2 of the electrically induced blink reflex and the air puff–induced blink reflex terminate in different subnuclei of the sensory trigeminal complex (Berardelli et al. 1985; Ndiaye et al. 2002). Peshori et al. (2001) repeatedly stimulated the SO nerve in healthy subjects between 20 and 80 yr of age, and they elicited blink oscillations. Moreover, these authors found that after age 40 yr, blink oscillations occur in blinking patterns spontaneously. We assume that aging of neurons in the sensory trigeminal complex may cause inconstant blinking and blink oscillations.

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