

Key Words

skin, pseudoexfoliative material, lectin, electron microscopical histochemistry, glycoconjugates

References

1. Spicer SS, Schulte BA. Diversity of cell glycoconjugates shown histochemically: A perspective. *J Histochem Cytochem.* 1992;40:1-38.
2. Schlötzer-Schrehardt U, Koca R, Naumann GOH, Volkholtz H. Pseudoexfoliation syndrome: Ocular manifestation of a systemic disorder? *Arch Ophthalmol.* 1992;110:1752-1756.
3. Umihira J, Nagata S, Nohara M, et al. Localization of elastin in the normal and glaucomatous human trabecular meshwork. *Invest Ophthalmol Vis Sci.* 1994;35:486-494.
4. Streeten BW, Gibson SA, Li Z-Y. Lectin binding to pseudoexfoliative material and the ocular zonules. *Invest Ophthalmol Vis Sci.* 1986;27:1516-1521.
5. Hietanen J, Tarkkanen A. Glycoconjugates in exfoliation syndrome: A lectin histochemical study of the ciliary body and lens. *Acta Ophthalmol (Copenh).* 1989;67:288-294.
6. Leatham A, Atkins N. Lectin binding to formalin-fixed paraffin section. *J Clin Pathol.* 1983;36:747.
7. Roth J, Bendayan M, Carlemalm E, Villiger W, Garavito M. Enhancement of structural preservation and immunocytochemical staining in low temperature embedded pancreatic tissue. *J Histochem Cytochem.* 1981;29:663-671.
8. Dark AJ, Streeten BW. Pseudoexfoliation syndrome. In: Garner A, Klintworth GK, eds. *Pathobiology of Ocular Disease.* New York: Marcel Dekker; 1982:1303-1320.
9. Streeten BW, Bookman L, Ritch R, Prince AM, Dark AJ. Pseudoexfoliative fibrilopathy in the conjunctiva: A relation to elastic fibers and elastosis. *Ophthalmology.* 1987;94:1439-1449.
10. Sharon N, Lis H. *Lectins.* London: Chapman and Hall; 1989:69-90.

Conjugacy of Spontaneous Blinks in Man: Eyelid Kinematics Exhibit Bilateral Symmetry

Michael W. Stava,* Mark D. Huffman,*
Robert S. Baker,*† Avrom D. Epstein,*†
and John D. Porter*‡

Purpose. To provide a quantitative description of the conjugacy of human eyelid movements during spontaneous blinks.

Methods. Eyelid movements occurring during spontaneous blinks were recorded bilaterally using a modification of the electromagnetic search coil technique. In off-line analyses, covariation of amplitude, peak velocity, and duration of blink down phases were determined for the two eyelids. Interocular differences in the timing of blink onset and offset, and time to peak velocity, also were evaluated.

Results. Human blink motor control systems act to link tightly the spatial and temporal characteristics of movements of the two eyelids. Data show that human sponta-

neous blinks are conjugate. Analysis of interocular covariation of blink amplitude, peak velocity, and duration yielded linear functions with high correlation coefficients. Interocular comparison of eyelid movement durations during blinks showed a particularly high correlation. There were negligible interocular differences in blink down-phase onset time, termination time, and time to peak velocity. A small percentage of blinks exhibited interocular differences in amplitude and peak velocity of >20%; however, even in these cases, blink duration remained tightly linked.

Conclusion. Spatial and temporal properties of eyelid movements occurring during spontaneous blinks are conjugate. These data support the hypothesis that a bilateral gating mechanism regulates blink duration. Elements downstream from the gate may differentially and unilaterally alter blink amplitude and peak velocity, but the duration of blinks remains time-locked for the two eyelids. *Invest Ophthalmol Vis Sci.* 1994;35:3966-3971.

Human blinks are controlled by the reciprocal activity of two skeletal muscles, the levator palpebrae superioris and the orbicularis oculi (for review, see Schmidtke and Büttner-Ennever¹). To execute the smooth eyelid movements seen during blink, the activity in these antagonistic muscles is tightly coordinated. Blinking requires coordination between two brainstem cranial motor nuclei: levator palpebrae superioris motoneurons lie in the caudal central subnucleus of the oculomotor nerve, whereas orbicularis oculi motoneurons are in a subdivision of the facial nucleus. The central pathways responsible for the coordinated, reciprocal activity of levator palpebrae superioris and

From the Departments of *Ophthalmology, †Neurology, and ‡Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, Kentucky. Supported in part by an unrestricted grant from Research to Prevent Blindness, Inc., New York, New York, by a grant from the Benign Essential Blepharospasm Research Foundation, Beaumont, TX, and by National Eye Institute grant EY10760.

Presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, May 1994.

Submitted for publication February 24, 1994; revised May 12, 1994; accepted May 23, 1994.

Proprietary interest category: N.

Reprint requests: Dr. Robert S. Baker, Department of Ophthalmology, E304 Kentucky Clinic, University of Kentucky Medical Center, Lexington, KY 40536-0284.

orbicularis oculi motoneurons are not yet understood. During maintenance of eyelid position, the levator palpebrae superioris is tonically active and the orbicularis oculi is silent.²⁻⁴ Upon initiation of blink, however, there is a concomitant inhibition of the sustained levator palpebrae activity and phasic activation of orbicularis oculi. The kinematics of blink termination show an initial rapid rise, followed by a slower, exponential return to the previous eyelid position. In blink termination, orbicularis oculi motoneuron activity ceases and the up phase of the blink is preceded by either burst-tonic or tonic activity in levator palpebrae motoneurons.⁴

Recent studies have demonstrated that eye and eyelid movements are tightly coupled.³ In all species studied, eyelid position is maintained at the same point relative to the pupil during gaze changes to optimize vision and protective reflexes. However, the bilateral coupling of eyelid movements and the mechanisms responsible for the establishment and maintenance of the conjugacy of blinks have received little attention. The interocular coordination of eyelid movements in blink may exhibit species differences. Organisms with binocular vision may be best served by conjugate blink control systems, whereas those species with little binocularity may benefit from independent control of the two eyelids. Species such as man with frontally oriented eyes and binocular vision would be better served by the bilateral coordination of eye and eyelid movement. Recent studies^{5,6} are consistent with this hypothesis, showing that the monkey, a frontally-eyed species, exhibits conjugate blinks. Conjugacy of eyelid movement would ensure protection of both eyes when either eye is stimulated, as in the trigeminally driven corneal blink reflex. Findings in several clinical conditions also support the notion of conjugate control of eyelid function.^{7,8}

Although the metrics of human blink movements have been addressed,^{2,3,9,10} there is limited information regarding the symmetry of movement of the two eyelids. This study quantified the spatial and temporal characteristics of movements of the eyelids occurring during spontaneous blinks in man.

MATERIALS AND METHODS. The bilateral symmetry of human blink metrics was studied using a modification of the magnetic scleral search coil technique, as described previously.^{3,5,10} The subjects were five adults, two men and three women (Table 1). Subjects displayed no ophthalmologic abnormalities other than mild, refractive errors, and they gave prior informed written consent to participate in the study. The human use protocol conformed to the Declaration of Helsinki and was approved by the Internal Review Board at the University of Kentucky Medical Center. Each subject was comfortably seated in a chair centered within a weak electromagnetic field. The

magnetic field was generated by a 6-foot Robinson field coil system (CNC Engineering; Seattle, WA). Head position was fixed, and fine wire coils (30 turns, 6 mm outer diameter, 160 mg, Teflon-coated copper wire) were taped to the lower margin of the upper eyelid at a position directly above the pupil. The coils did not appear to hinder eyelid movement. Angular rotation of the coils within the weak magnetic field generated a voltage proportional to eyelid position. Coils were calibrated frequently by rotation through known angles and yielded a linear voltage output across a $\pm 40^\circ$ range. Spontaneous blinks were recorded while subjects watched television from a distance of 60 cm. Watching television was the only instruction given subjects; sporadically, each subject was instructed to blink. During recording sessions, which lasted approximately 40 minutes, eyelid position signals were amplified and filtered (500 Hz cut-off), digitized, sampled at 500 Hz per channel, and were A/D converted with 12-bit precision (resolution was 0.02°). Sampling rate was chosen on the basis of Fourier analysis, which indicated that 90% of blink energy lies below 90 Hz.³ Data were stored on an IBM-compatible 80386 computer equipped with digital oscilloscope software for later off-line analysis. Investigators analyzed the first 50 blinks from each subject by marking right and left eyelid blink tracings with cursors to identify blink amplitude, peak velocity, and duration independently for each eyelid (Fig. 1A). One author analyzed all data to standardize determination of movement onset (beginning of downward deflection of the position trace) and offset (leveling of the position trace in the trough of the blink). For comparison of interocular differences in blink kinematics, we defined the terms *amplitude gain* as left eyelid amplitude \div right eyelid amplitude and *peak velocity gain* as left eyelid peak velocity \div right eyelid peak velocity.

RESULTS. The profile of blinks measured with the search coil technique in this study appeared similar to those seen with other techniques.¹¹ Ballistic movements, such as blinks, exhibit a characteristic relationship between amplitude, peak velocity, and duration known as the main sequence. For the data collected in these studies, the inter-subject variability was low. Although some subjects presented, on average, larger spontaneous blinks than others (Table 1), the main sequence relationship seen in Figure 2A provided a close fit for data from all subjects. Similarly, measures of blink conjugacy were similar for all subjects, and the data reported represent a pooling across subjects.

During spontaneous blinks, the human eyelid position traces exhibited a rapid down-phase deflection corresponding to eyelid closure and a slower up-phase deflection corresponding to eyelid opening (Fig. 1). Blink down phases were fairly stereotypical, whereas

TABLE 1. Human Main Sequence Data for Spontaneous Blinks

Subjects			Amplitude (deg)		Peak Velocity (deg/sec)		Duration (msec)	
Number	Age	Sex	OS	OD	OS	OD	OS	OD
1	25	F	22.77 ± 4.71	24.45 ± 5.31	573.60 ± 104.73	465.10 ± 144.38	121.02 ± 26.70	111.39 ± 25.23
2	27	M	33.15 ± 3.52	31.18 ± 3.46	812.60 ± 103.44	730.80 ± 131.77	109.43 ± 24.07	104.73 ± 19.02
3	43	F	34.81 ± 9.48	38.34 ± 8.96	868.90 ± 244.78	1107.20 ± 270.73	95.59 ± 30.29	92.91 ± 25.51
4	22	F	19.18 ± 7.02	18.43 ± 6.76	481.90 ± 203.58	608.60 ± 205.97	83.21 ± 27.41	74.46 ± 26.40
5	28	M	42.37 ± 10.35	38.51 ± 10.26	999.50 ± 232.55	897.10 ± 213.09	114.22 ± 33.60	106.75 ± 34.07

Table reports mean value ± 1 standard deviation for left (OS) and right (OD) eyelids.

blink up phases exhibited more variability in both peak velocity and duration. Although most spontaneous blinks display a rapid termination followed by an exponential return to the base line, occasional blinks exhibited a variable duration plateau between the down and up phases. Across the five subjects, mean

blink amplitude was $30.54^\circ \pm 11.2^\circ$ (SD) (total amplitude range for single blinks was 3.7° to 59.7° ; Table 1).

As seen in other human blink studies,^{3,10} amplitude and peak velocity were tightly correlated ($r = 0.88$) and exhibited a linear relationship ($y = 20.9x + 111.6$) (Fig. 2A). Furthermore, as in previous blink studies, duration was found not to be a good predictor of amplitude.³ Data from this study confirm findings^{3,10} that blink amplitude and duration were not tightly correlated ($r = 0.36$).

The central finding of this study was that the kinematics of eyelid movements in spontaneous blinks are highly conjugate. Analysis of the covariance of blink amplitudes generated by the two eyelids indicates that they are tightly linked ($r = 0.94$) (Fig. 2B). Amplitude gain values for most spontaneous blinks (89%) fell in the range of 0.8 to 1.2 (a value of 1 indicates exact symmetry) (Table 2, Fig. 3). Linear relationships also were seen when comparing the covariation of both peak velocity and duration across the two eyelids ($r = 0.82$, $r = 0.94$, respectively) (Figs. 2C, 2D). Although peak velocity gain values exhibited conjugacy, they were more variable than the values obtained for amplitude gain (only 63% of spontaneous blinks exhibited velocity gains between 0.8 and 1.2) (Table 2, Fig. 3). The time from blink onset to peak velocity was tightly correlated for the two eyelids, occurring approximately halfway through the down phase of the lid movement. Blink onset and termination also displayed negligible differences (onset difference = 6.86 ± 6.49 msec; offset difference = 4.80 ± 7.36 msec).

Some blinks, however, showed significant interocular differences in both amplitude and peak velocity (Fig. 1B). Approximately 11% of blinks exhibited amplitude gains outside the range of 0.8 to 1.2, whereas 37% of the blinks had peak velocity gains outside this range. Despite the significant interocular differences in amplitude and peak velocity found in such blinks, eyelid movement duration remained tightly matched for the two eyelids.

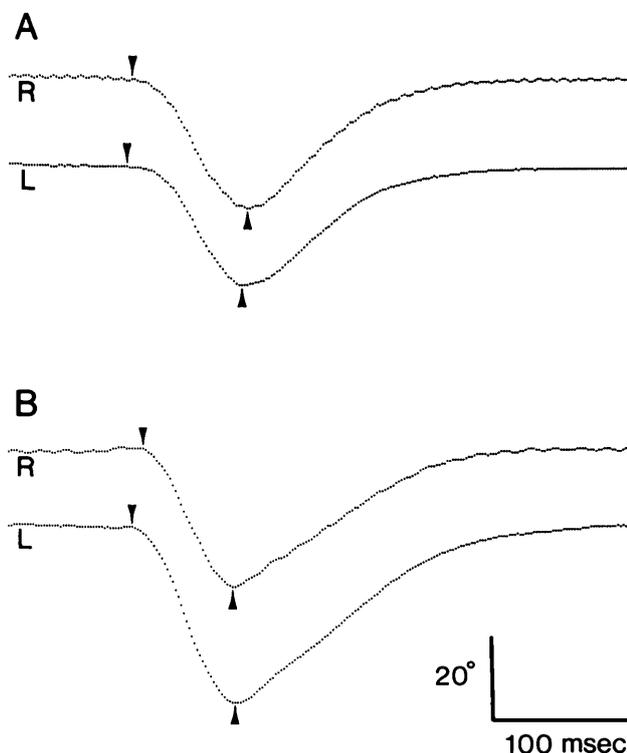


FIGURE 1. Representative eyelid movement traces during spontaneous blinks, measured with the search coil technique. Down arrows indicate blink down-phase onset; up arrows indicate down-phase offset. (A) Single blink record illustrating conjugacy of the metrics of the left and right eyelids (right eyelid amplitude = 27.2° , peak velocity = $660^\circ/\text{sec}$; left eyelid amplitude = 29.4° , peak velocity = $780^\circ/\text{sec}$). (B) Blink record displaying dysconjugacy of blink metrics in the left and right eyelids. Note that despite the lack of correspondence in blink amplitude and peak velocity (right eyelid amplitude = 31.5° , peak velocity = $780^\circ/\text{sec}$; left eyelid amplitude = 40.8° , peak velocity = $975^\circ/\text{sec}$), there is still a tight correspondence in movement onset and offset times.

DISCUSSION. Studies have used magnetic search coil techniques to evaluate eyelid movements

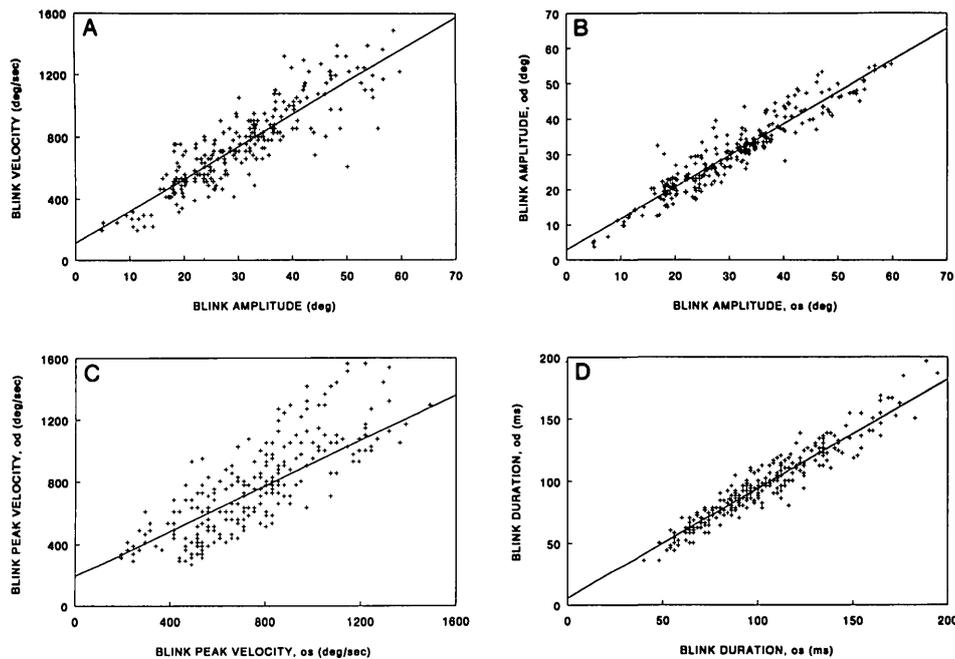


FIGURE 2. Graphs illustrating the blink main sequence (A) and the covariation of blink metrics in the two eyelids (B, C, D) for all subjects. (A) Main sequence relationship of amplitude versus peak velocity. (B) Covariation of right eyelid amplitude versus left eyelid amplitude. (C) Covariation of right eyelid peak velocity versus left eyelid peak velocity. (D) Covariation of right eyelid duration versus left eyelid duration.

monocularly, thereby providing quantitative descriptions of human blink kinematics.^{3,10} Present data are in close agreement with these earlier monocular data, confirming their stereotypical nature and indicating that blinks are best described by the main sequence relationship between amplitude and peak velocity. Blink duration has not been shown to be a reliable predictor of amplitude. By contrast, the coordination of movements of the two eyelids, or the *conjugacy of blink*, has received only limited attention.⁵ This study provides the first detailed quantitative data regarding the symmetry of human eyelid movements in blink. Most spontaneous blinks in man exhibit covariation of the amplitude, peak velocity, and duration characteristics of the two eyelids. Although a minority of blinks may show significant interocular differences in amplitude, with corresponding differences in peak ve-

locity, blink duration remains tightly matched for the two eyelids.

These findings are consistent with data obtained in a monkey model of eyelid paresis.⁶ Despite the amplitude asymmetries induced by unilateral injections of botulinum toxin into the orbicularis oculi, adaptive adjustments of the duration of blink in the paretic eyelid were accompanied by corresponding extensions of blink duration in the nonparetic eyelid. Taken together, these data support the idea that blink systems maintain conjugacy by matching the duration of

TABLE 2. Human Blink Gain for Spontaneous Blinks

Subjects			Gain	
Number	Age	Sex	Amplitude	Peak Velocity
1	25	F	0.94 ± 0.11	1.28 ± 0.21
2	27	M	1.07 ± 0.06	1.14 ± 0.18
3	43	F	0.91 ± 0.11	0.78 ± 0.10
4	22	F	1.05 ± 0.14	0.78 ± 0.15
5	28	M	1.12 ± 0.11	1.13 ± 0.16

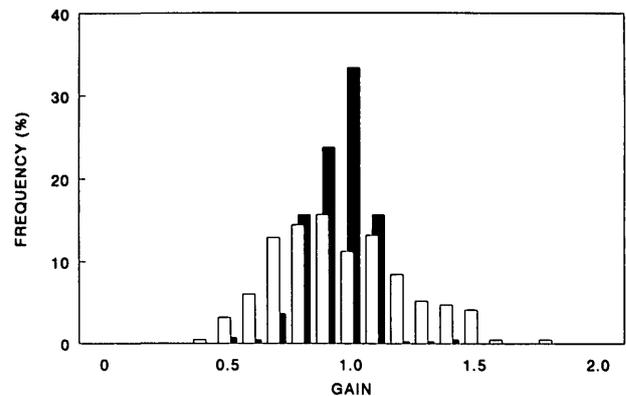


FIGURE 3. Histogram depicting the distribution of blink amplitude gains (solid bars) and peak velocity gains (open bars) for all subjects. Data indicate that, although most blinks are of similar amplitude and peak velocity, some blinks are dysconjugate.

movements of the two eyelids. It is clear that a thorough understanding of mechanisms that mediate the binocularity of blink will be vital to the interpretation and treatment of eyelid movement disorders.

The conjugacy of blink may exhibit species differences based upon placement of the eyes, frontal versus lateral. Presumably, frontal-eyed species adaptively developed blink conjugacy to aid binocular vision. Conjugacy of blink is adaptive for frontal-eyed species because a threat to one eye often requires a bilateral response. Blink conjugacy also may be important for organisms with binocular vision in mediating the brief suppression of vision that occurs with each eyelid closure.¹² The colocalization of levator palpebrae motoneurons that serve both eyelids in primates (in a midline caudal central subnucleus of the oculomotor nuclear complex)¹³ facilitates their symmetric inhibition during blink initiation. By contrast, lateral-eyed species lack a caudal central nucleus and the corresponding colocalization of levator motoneuron pools. In addition, these species have reduced binocular vision and may be better served by disconjugate protective reflexes. Thus, the differential localization of levator palpebrae motoneurons in frontal-eyed versus lateral-eyed species¹³ directly correlates with differences in dependence–independence of eyelid movement and the extent of binocular vision.

The neural mechanisms responsible for maintaining conjugacy of eyelid movements in blink may be tightly linked to those controlling conjugate eye movements. Several studies^{7,8} suggest that Hering's law of equal innervation for eye movements also may apply to blink. Normal subjects can elevate one eyelid independently, but only by imposing asymmetric frontalis activity not typically seen in the control of eyelid movements.⁷ Similarly, in patients with unilateral ptosis, retraction of the contralateral lid is observed. This phenomenon is thought to reflect adaptive increases in levator palpebrae gain in the ptotic lid that, because of principles of equal innervation, also is expressed in the levator of the normal eyelid. Covering or manually raising the ptotic lid eliminates the retraction, suggesting that visual feedback plays an important role in the modulation of the conjugacy of eyelid movements in the same way that it plays a role in the adaptive regulation of eye alignment and conjugacy of eye movements. Collectively, these data argue for the bilateral, coordinated relaxation of the levator palpebrae superioris at the onset of blink and coordinated resumption of levator palpebrae superioris activity with blink termination. Mechanisms responsible for the coordinated excitation of orbicularis oculi motoneuron pools are unclear.

Blink conjugacy might be established and maintained by the same population of brainstem omnipause neurons that function in eye movements. For eye saccades, separate pools of medium lead burst

neurons provide the appropriate excitatory (or inhibitory) drive that specifies the duration of high-frequency burst activity (or pause duration in the antagonist) in oculomotor motoneurons, thereby determining the duration of the saccade. Omnipause neurons tonically inhibit medium lead burst neurons, and, by pausing, they allow the occurrence of a saccade. Thus, omnipause cells serve an important role in modulation of the temporal characteristics of the saccade by precisely gating the discharge duration of the medium lead burst neurons.¹⁴ Although omnipause neurons lack directional selectivity, which must be encoded by other premotor signals, they do specify the movement *duration* for both eyes. Recent studies have implicated this same pool of omnipause neurons in gating blink.¹⁵ Although various excitatory signals to a putative pool of blink premotor burst neurons are necessary to determine the recruitment of facial nucleus motor units (which, in turn, specifies the amplitude and peak velocity of blink), it is likely that the omnipause signal gates the onset and offset of burst neurons that drive blink *bilaterally*. This hypothesis predicts that, although the compensatory changes in blink metrics observed after unilateral paresis of the orbicularis oculi may include interocular differences in both static eyelid position and amplitude-peak velocity relationships, the temporal characteristics of blink will remain closely matched for the two eyelids.

Key Words

blink, conjugacy, human, eyelid, orbicularis oculi

Acknowledgments

The authors thank Dr. Elmar Schmeisser and Mr. Chuck Chase for technical assistance in adapting a signal digitizer and coil system for the eyelid movement studies; Drs. Paul May and Larry Mays for providing helpful insights into the neural control of blink; and Dr. Craig Evinger for invaluable consultation on the collection and analysis of human blinks.

References

- Schmidtke K, Büttner-Ennever JA. Nervous control of eyelid function: A review of clinical, experimental and pathological data. *Brain*. 1992;115:227–247.
- Evinger C, Shaw MD, Peck CK, Manning KA, Baker R. Blinking and associated eye movements in humans, guinea pigs, and rabbits. *J Neurophysiol*. 1984;52:323–339.
- Evinger C, Manning KA, Sibony PA. Eyelid movements: Mechanisms and normal data. *Invest Ophthalmol Vis Sci*. 1991;32:387–400.
- Fuchs AF, Becker W, Ling L, Langer TP, Kaneko CRS. Discharge patterns of levator palpebrae superioris motoneurons during vertical lid and eye movements in the monkey. *J Neurophysiol*. 1992;68:233–243.
- Porter JD, Stava MW, Gaddie IB, Baker RS. Quantitative analysis of eyelid metrics reveals the highly stereo-

- typed nature of monkey blinks. *Brain Res.* 1993; 609:159–166.
6. Porter JD, Baker RS, Stava MW, Gaddie IB, Brueckner JK. Types and time course alterations induced in monkey blink movements by botulinum toxin. *Exp Brain Res.* 1993;96:77–82.
 7. Gay AJ, Salmon ML, Windsor CE. Hering's law, the levators and their relationship in disease states. *Arch Ophthalmol.* 1967;77:157–161.
 8. Schechter RJ. Ptosis with contralateral lid retraction due to excessive innervation of the levator palpebrae superioris. *Ann Ophthalmol.* 1987;10:1324–1328.
 9. Becker W, Fuchs AF. Lid-eye coordination during vertical gaze changes in man and monkey. *J Neurophysiol.* 1988;60:1227–1252.
 10. Guitton D, Simard R, Codère F. Upper eyelid movements measured with a search coil during blinks and vertical saccades. *Invest Ophthalmol Vis Sci* 1991; 32:3298–3305.
 11. Snow BJ, Frith RW. The relationship of eyelid movement to the blink reflex. *J Neurol Sci.* 1962;91:371–377.
 12. Manning KA, Riggs LA, Komenda JK. Reflex eyeblinks and visual suppression. *Percept Psychophysiol.* 1983; 34:250–256.
 13. Porter JD, Burns LA, May PJ. Morphological substrate for eyelid movements: Innervation and structure of primate levator palpebrae superioris and orbicularis oculi muscles. *J Comp Neurol.* 1989;287:64–81.
 14. Fuchs AF, Kaneko CRS, Scudder CA. Brainstem control of saccadic eye movements. *Ann Rev Neurosci.* 1985;8:307–337.
 15. Mays LE, Morriss DW. Stimulation of pontine omnipause neurons inhibits eye blinks. ARVO Abstracts. *Invest Ophthalmol Vis Sci.* 1994;35:1547.