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Residual eye-movements in macaque and their effects on visual responses of neurons

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Abstract

We recorded continuously, with high precision, the positions of the eyes in anesthetized macaque monkeys prepared for physiological recording. Most recordings were made after the infusion of muscle relaxant to immobilize the eyes; in some cases we also were able to record eye position for periods before the eyes were immobilized. In all monkeys, the eyes moved continuously by as much as 0.5 deg over a 10-min sampling period. The average distance moved was proportional to the square root of the sampling period, as would be expected from a random walk. The movements had three distinct components: slow drifts, and two rhythms driven by the pulse and respiration. The rhythmic movements occurred only under paralysis: they were not discernible in measurements made before the infusion of muscle relaxant. The movements of the eye in the paralyzed animal can have substantial effects on the measured physiological characteristics of neurons. For excursions in the midrange of those we observed, a neuron's sensitivity to a spatial frequency of 10 cycle/deg might be underestimated by as much as a factor of three, depending on the method by which responses were averaged. We show how the effects of eye-movements can be mitigated by appropriate data analysis.

Keywords: Eye-movements, Paralysis, Vision

Introduction

Muscle relaxants are routinely used to immobilize the eyes in experiments to study the receptive fields of visual neurons in anesthetized animals. Despite this, the eyes move. These movements have been studied systematically in the cat (Rodieck et al., 1967; Barlow et al., 1974; Linsenmeier & Hertz, 1979). Accompanied by cervical sympathectomy (the cat, unlike the monkey, has sympathetic innervation of orbital muscle), muscle relaxants can reduce eye-movements to *ca.* 10 min arc over periods of minutes (Rodieck et al., 1967; Linsenmeier & Hertz, 1979). Because the cat has large receptive fields, these residual movements will seldom be consequential for visual physiology.

Receptive fields in the monkey's visual system are much smaller than those in the cat, and physiological measurements are correspondingly more vulnerable to small eye-movements. In making unpublished measurements of the optical linespread function of the monkey's eye using the method of Robson and Enroth-Cugell (1978), A.M. Derrington and P. Lennie found that, even with the eye firmly stabilized by attachment to a ring, movements associated with pulse and respiration occurred with amplitude large enough to interfere with the measurements. In experiments using stationary images to study neurons in macaque V1, Müller et al. (2001) found that eye-movements often interfered with measurements. Roorda et al. (2001), in accumulating a series of high-resolution photographs of the photoreceptor mosaic in the living eye of macaque, found that eye-position often changed from one photograph to the next. In this paper, we describe measurements of these eye-movements in the macaque monkey under conditions likely to prevail during normal physiological recording, and we illustrate the potential effects of the movements on the characterization of neurons. We then show how these effects can be mitigated.

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Methods

Male monkeys (*M. fascicularis*) weighing between 3.75 and 5.45 kg were prepared for single-unit recording, as described in Müller et al. (2001).

A well-fitting opaque contact lens, to which a fragment of cover slip had been glued (total weight 25 mg), was placed on one eye. Several minutes were allowed to elapse while the lens became firmly attached to the eye. The beam from a diode laser (635 nm) was reflected off the coverslip on to the surface of UDT DLS-4 position sensing detector placed about 14 cm in front of the eye. This detector, coupled to its amplifier, provided continuous output voltages proportional to the *X* and *Y* positions of the centroid of the

laser spot on its 4 × 4 mm sensing surface. The device had a relative accuracy limited only by noise—in practice it could resolve a 2-sec arc change in gaze. *X*- and *Y*-position signals were low-pass filtered (30 Hz) then sampled continuously by a Macintosh computer at 100 Hz with 16-bit resolution, and saved for later analysis. We usually recorded eye-position for 10 min.

We recorded the position of one eye in each of nine anesthetized monkeys. In five of these, the recordings were made shortly after the monkey had been paralyzed with vecuronium bromide (Norcuron; loading dose of 50 μg/kg, followed by a continuous infusion at 100 μg/kg/h); in the remaining animals measurements were made 3 or 4 days later, when we had finished single-unit recording. For two animals, measurements were made both at the beginning and the end of recording. We found no difference in the character of the measurements made early and late, and have not distinguished these in the Results. In several monkeys, we also attempted to record eye-position before the infusion of muscle relaxant; we were able to obtain adequate records of eye-position in only two because frequent large eye-movements tended to move the laser spot off the sensor.

Results

In all monkeys, the eyes moved throughout the course of the measurement. Figs. 1A–1C show, for eyes of three monkeys, *X*–*Y* plots of eye-position at 0.25-s intervals for 10 min.

The eyes moved during the recording period, but generally without systematic progression (the movement shown in Fig. 1B—a steady progression of the eye up and to the left—was exceptional). This is easily seen in Figs. 1D, 1E, and 1F (counterparts to Figs. 1A, 1B, & 1C) which display the horizontal and vertical positions of the eyes throughout the recording period. The excursions of the eyes shown in Fig. 1 represent the range encountered, from less than 0.1 deg for the eye represented in Figs. 1A and 1D to over 0.5 deg for the eye represented in Figs. 1C and 1F.

We can obtain a different view of the eye-movements by looking at the average changes in position during sampling periods of different durations. Fig. 2A shows the mean change in position of each of seven eyes in different monkeys during independent measuring periods from 0.5 s to 256 s. Although the absolute excursions differ among eyes, the trends are the same, with the average distance traveled by the eye being approximately proportional to the square root of the sampling period. This is the behavior expected from a random walk. The distances moved by some eyes (e.g. the one that moved most, shown in the uppermost trace) are very well characterized by this rule. Among eyes that moved less, the departures from the square-root relationship are principally at short sample durations, during which small oscillatory eye-movements are dominant (see below). Fig. 2B shows the standard deviation of distances moved. This also grows approximately as the square root of the sampling period (i.e. the standard deviation of eye-position grows in proportion to the average distance traveled), as would be expected from a random walk (Berg, 1983). There must be upper limits to the sample time and distance over which the square-root relationship holds, but for most eyes these limits exceed the ranges studied here.

The major excursions of the eyes occur as drifts. In addition to these large and generally unsystematic movements, there are two less prominent periodic components, one associated with respiration, and another associated with the pulse. These are most evident in the texture of the traces in Fig. 1E. An enlarged portion of the horizontal trace from Fig. 1E is reproduced in Fig. 3, together with a sample from another eye. The trace in Fig. 3A shows a prominent modulation of about 0.8 min arc at the frequency of the pulse (102/min) with an additional less prominent modulation (0.3 min arc) at the frequency of respiration (24/min). For the eye represented in Fig. 3B, movements produced by the pulse (134/min) were considerably smaller than for the other eye, while those produced by respiration (24/min) were of about the same amplitude.

Our method of measuring eye-position can in principle confuse small rotations and large fore–aft movements of the globe. We saw no sign of fore–aft movements, but had they existed with discernible amplitude, say 0.2 mm, they would have been registered as *X*- or *Y*-rotations of 0.0001 deg. The amplitude of the average pulse modulation produced a beam displacement at the sensor equivalent to a fore–aft movement of about 1.9 mm, which would have been clearly evident. We therefore conclude that our measurements record real rotations of the eye.

For the seven eyes on which we made measurements, the average depth of the modulations due to respiration and pulse were 1.0 min arc and 1.4 min arc, respectively. The phases of the horizontal and vertical components of pulse modulation were (with one exception) almost identical, so the eye oscillated along a line (see Fig. 1B). The rhythmic eye-movements caused by respiration or pulse, were they present in the normal awake animal, would be large enough to be visually troublesome. Recordings of human eye-movements during fixation show no indication of perturbations caused by respiration or pulse (Ditchburn & Ginsborg, 1953), neither do measurements of eye-position made routinely during physiological recordings from awake, behaving monkeys. This prompted us to ask if they resulted from anesthesia or paralysis. We examined how paralysis affected the respiration- and pulse-induced eye-movements by comparing the fine-structure of eye-movements recorded before and during paralysis, in monkeys in which anesthesia was maintained throughout at the relatively light level used for single-unit recording. It was particularly hard to record eye-position without paralysis, for frequent large eye-movements deflected the measuring beam off the sensor surface (1.7 deg × 1.7 deg). As a result, we obtained satisfactory recordings of adequate duration from only two animals. Fig. 4 shows two segments of the vertical position record from the left eye of one animal, one (A) obtained before and the other (B) during paralysis.

The two traces differ in their temporal fine-structure: the one obtained before paralysis is characterized by small and unsystematic drifts among periods of complete stability; the one obtained under paralysis has the clear rhythms of pulse and respiration. The different character of the two records is easily seen in their amplitude spectra (Figs. 4C & 4D): within the frequency range examined (0.017 Hz to 5 Hz, limited by the duration of relatively stable eye-position in the unparalyzed animal), movements of the unparalyzed eye contain little power at any frequency; those of the paralyzed eye contain more power at all frequencies, with notable spikes at 0.41 Hz (respiration) and at 1.67 Hz and higher harmonics (pulse).

Since paralysis promotes the rhythmic eye-movements, we wondered if the eye could be made resistant to them by stabilizing it. We explored this informally in one monkey. We mounted a metal rod horizontally in the stereotaxic frame on an axis parallel to the ear bars, and adjusted it so that its end just touched the lateral canthus, to which it was then attached securely with cyanoacrylate adhesive. This eliminated the drift but did not reduce the amplitude of the rhythmic movements. The resistance to stabilization was observed earlier by A.M. Derrington and P. Lennie while making

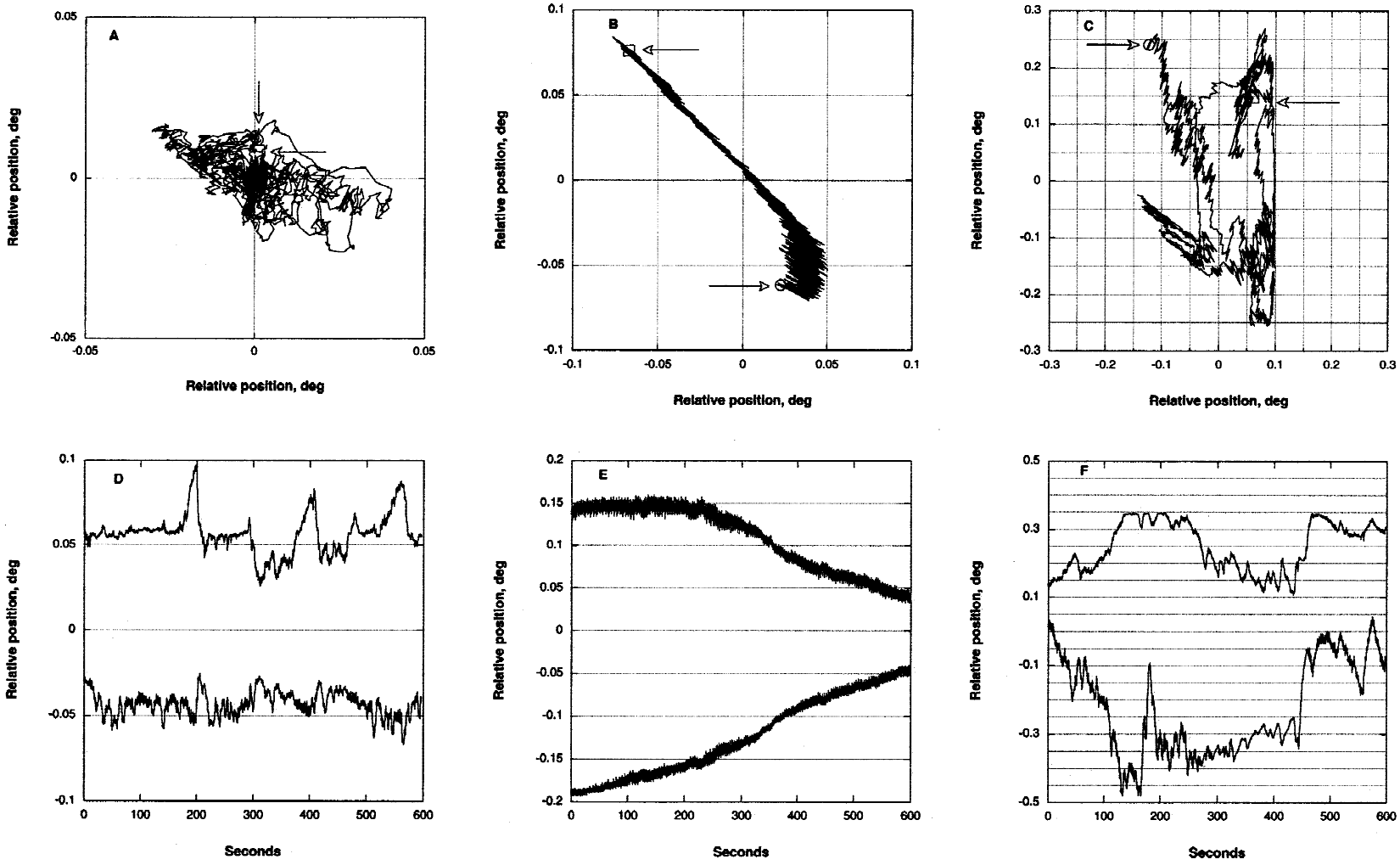

Fig. 1. Eye-position measured at 0.25-s intervals over a 10-min period under paralysis. A,B,C: Plots of horizontal and vertical position for each of three eyes in different monkeys. Each plot shows position in relation to the mean position during the sampling period. Starting and ending positions of the eye are marked by an open circle and a square, respectively (indicated by arrows). Scales differ: grid lines on each plot are spaced at 0.05 deg. The eye in A moved least among those studied; the eye in C moved most. D,E,F: Counterpart plots to those in A–C showing eye-position over time. Horizontal position (upper trace; positive is rightward movement) and vertical position (lower trace; positive is upward movement), both in relation to an arbitrary reference.

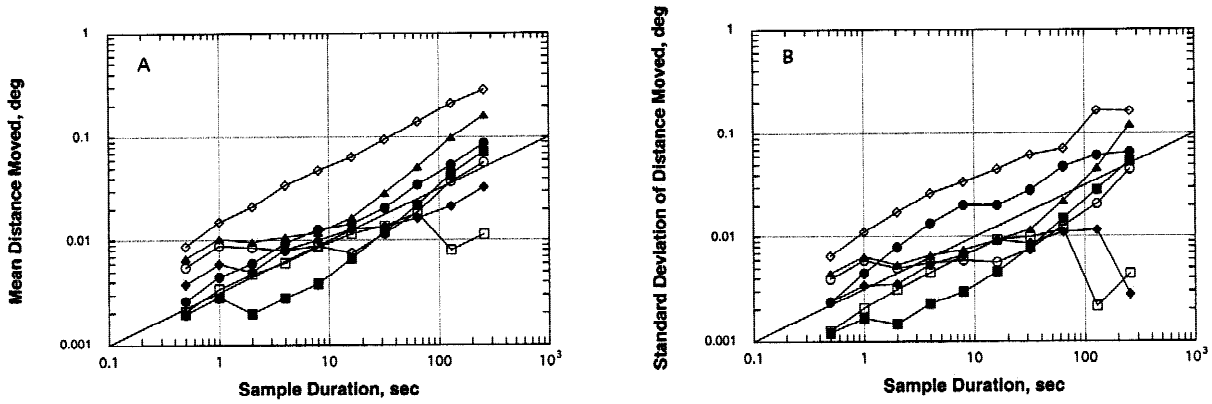

Fig. 2. Distance moved as function of measurement time for seven eyes. A: Average distance moved over sampling periods from 0.5 s to 256 s. The eyes represented in Fig. 1 are identified by open symbols (squares, 1A,C; circles 1B,E; & diamonds, 1C,F). The straight line drawn on the graph shows the form of the square-root relationship expected from a random walk. B: Standard deviation of the distance moved over sampling periods from 0.5 s to 256 s. Other details are as for A.

unpublished measurements of the optical linespread function of the monkey's eye.

Discussion

Our measurements show that monkeys' eyes move continuously even under systemic paralysis. In different animals, the eye-movements differ in scale, but have several features in common. The movements are dominated by slow drifts, on top of which are rhythmic movements that follow respiration and pulse. Rhythmic eye-movements caused by respiration have been observed in paralyzed cats (Robson & Enroth-Cugell, 1978), but have not been characterized. Eye-movements that track the pulse have not been characterized before.

Origin of movements

The rhythmic movements driven by pulse and respiration occur only when there is no muscle tone, and probably reflect the buffeting of the eye by the arterial pulse, and independently by the respiration. The pulse movements presumably follow the underlying expansion and contraction of the arterioles in the orbit. Movements that follow respiration reflect increases and decreases in venous pressure that accompany inflation and deflation of the chest.

The larger drifts of the eyes are less easy to understand, and we wondered if these might be driven by the smaller rhythmic movements. This could happen if there were static frictional forces to be overcome, and the small impulsive forces tended to propel the eye in a particular direction, resulting in ratchet-like movement. If in

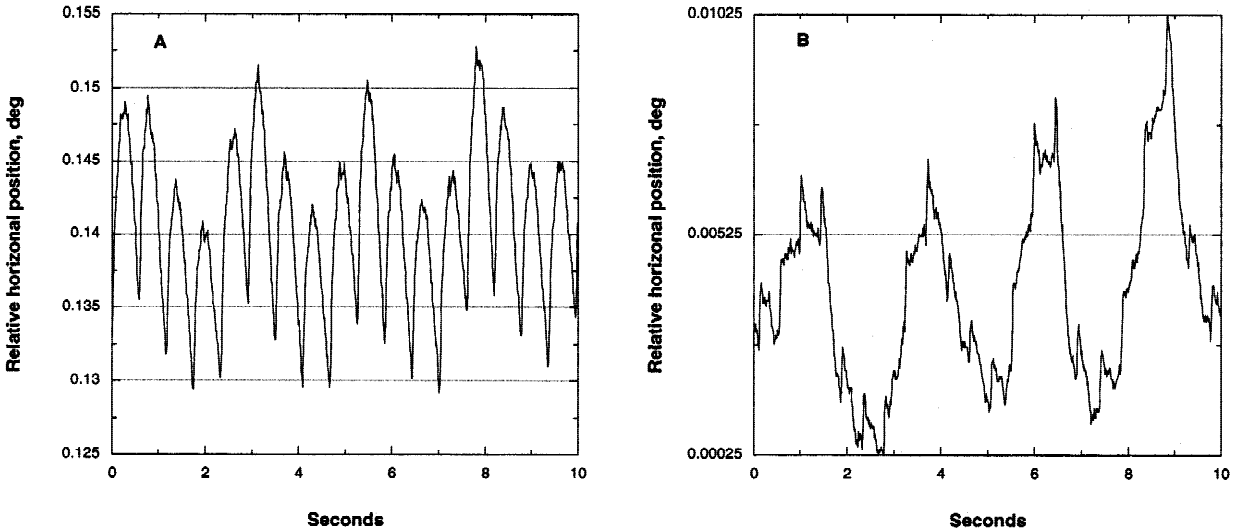

Fig. 3. Rhythmic eye-movements associated with the pulse and respiration. A: Horizontal eye-position measured over 10 s, showing periodicity with the pulse (102/min) and respiration (24/min). This is the eye represented in Fig. 1 (B,E). B: Same as A, for another eye from a different monkey. In this case, the pulse (134/min) is less prominent, the respiration (24/min) more so. Grid lines on both graphs are spaced at 0.05 deg.

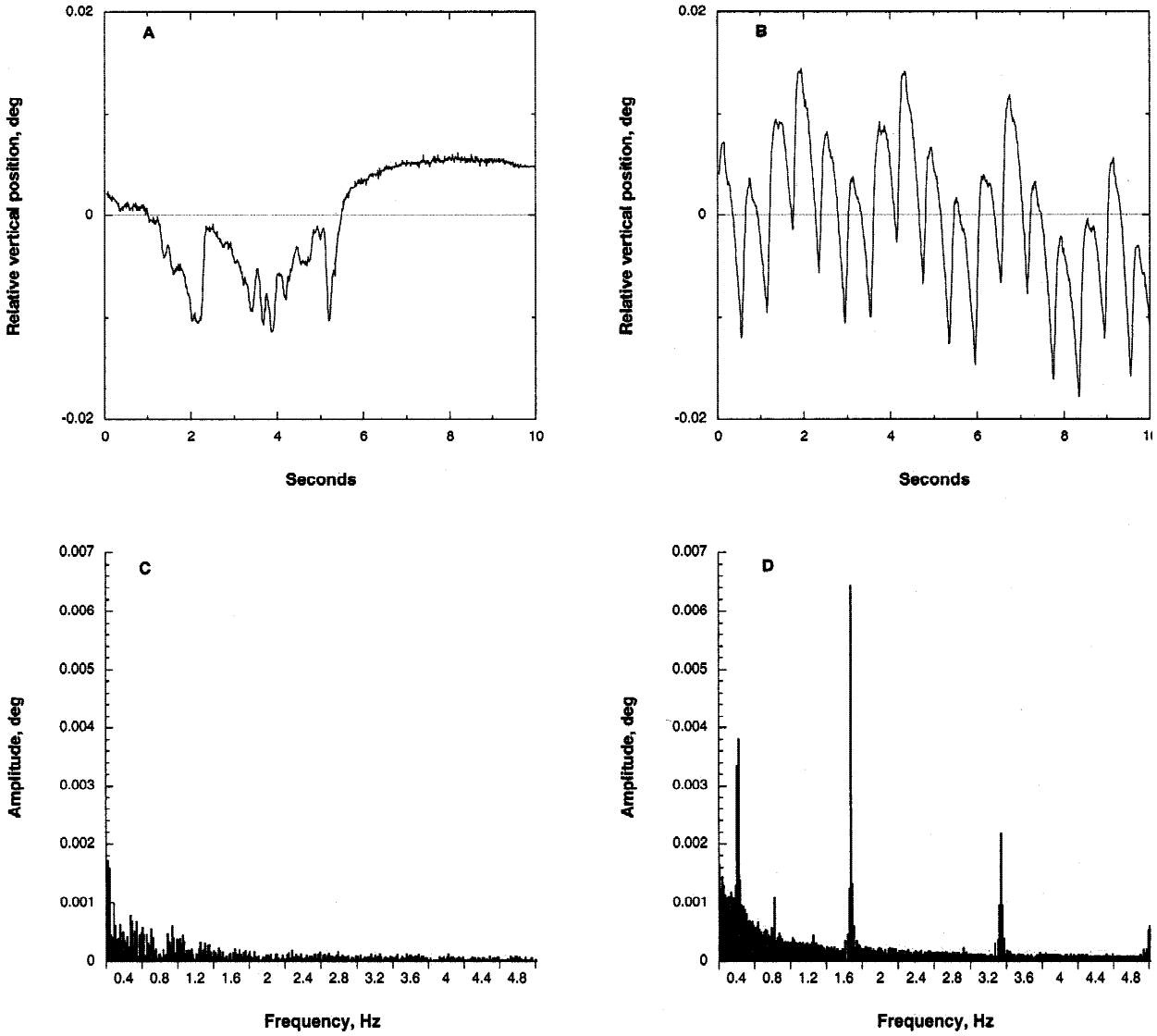

Fig. 4. Comparison of rhythmic eye-movements in unparalyzed and paralyzed eye. A: Horizontal eye-position record over 10 s without paralysis. B: Same as A, except eye-position was recorded after administration of muscle relaxant. C,D: Amplitude spectra of eye-position records in A and B, respectively.

fact the rhythmic movements drive drifts, we should expect the directions of the drifts to be correlated with the directions of the rhythmic movements.

To examine the correlations among the three types of movements, we filtered eye-position records in two frequency bands to isolate respiratory- and pulse-dependent movements, and also convolved the records with a temporal Gaussian filter to isolate the slow drifts. From each filtered record, we recovered the direction in which the eye moved from one sample point to the next. We then examined the correlations over time among the directions of the three kinds of movements. For the three eyes on which we undertook this analysis (those whose movements are shown in Fig. 1), the directions of the pulse- and respiration-dependent components of movement were well correlated in two (Figs. 1B & 1E, and 1C & 1F), but less well correlated in the third (Figs. 1A & 1D), in which the absolute amplitude of movement was smaller. The slow drifts were generally well correlated with the rhythmic movements in one eye (Figs. 1B & 1E), somewhat less well correlated for a second (Figs. 1C & 1F) and poorly correlated for the third (Figs. 1A & 1D).

The ratchet model can account for slow drift in the direction of the rhythmic movements, but it does not explain movements in other directions, or why the direction changes, sometimes fairly abruptly, over time.

Effects on characterization of receptive fields

Physiological observations show neurons in the lateral geniculate nucleus (LGN) (Derrington & Lennie, 1984; Blakemore & Vital-Durand, 1986; Crook et al., 1988) and cortex (De Valois et al., 1982) capable of resolving spatial frequencies higher than 20 cycle/deg when gratings are imaged through the eye's optics. Much higher spatial frequencies can be resolved when the optics are bypassed and gratings are formed as interference fringes

directly on the retina (McMahon et al., 2000). How physiological measurements might be affected by the kinds of eye-movements characterized here will depend very much on the particular procedures used in making the measurements. Since the probable excursion of the eye during the course of a measurement depends on the time over which the measurement is made (Fig. 2), one needs to know something about the method used to accumulate the response to a particular stimulus.

Consider an experiment in which we record discharge continuously for 400 s, during which time we present ten different gratings, each for a total of 40 s. The usual practice would be to present the grating in multiple epochs, say 20 of 2 s, each interleaved randomly with the presentations of other gratings from the set. The samples of response to a particular grating are gathered over a period of 400 s, during which the eye might have ranged over a considerable distance. If the neuron is sensitive to the spatial phase of the grating (e.g. a ganglion cell or LGN cell, or a simple cell in cortex), the changes in eye-position will cause variations in response amplitude that will vary with the spatial frequency of the grating. We estimated the potential effects of this using the three sets of measurements in Fig. 1. We simulated an experiment that delivered ten different gratings, each for 20 periods of 2 s, randomly interleaved. Each grating was in the optimal spatial phase for the initial position of the eyes, and lay in an orientation orthogonal to the direction of greatest excursion of the eyes. For each trial, we calculated the response expected from the particular phase alignment of the stimulus on the receptive field, then for each of the different gratings averaged the responses across all trials on which it had been presented. This is what one would do to construct a poststimulus time histogram. Fig. 5A shows, as a function of spatial frequency, the relative amplitude of response that would be measured in the face of the eye-movements shown in Fig. 2. This plot assumes a neuron whose underlying responsivity is the same at all spatial frequencies. For the eye that moved the most, the reduction in apparent amplitude is substantial even for a spatial frequency as low as 2 cycle/deg. The fluctuations in the curves at higher spatial frequencies reflect the fact that the change in eye-position from trial to trial is large in relation to the period of the grating, whose relative phase will then change randomly.

Fig. 5A shows that eye-movements can lead to a substantial underestimate of a neuron's responsivity to stimuli containing moderate to high spatial frequencies. There is no good solution to this problem if the experiment employs stationary stimuli. However, if stimuli move or are temporally modulated, and the responses can be phase aligned before analysis, or each can be analyzed by a phase-independent measure (e.g. the amplitude of the Fourier component at the appropriate frequency), measurements can be made robust against the kinds of eye-movements encountered in paralyzed monkeys. Fig. 5B shows the results of the same simulations as in Fig. 5A, but this time with relative amplitude of response derived by extracting the amplitude independently on each trial and then averaging the amplitudes over all trials for each grating. This procedure is susceptible to eye-movements only to the extent that the eye moves during a single trial. Since a trial lasts a short time (in this case 2 s), responsivity is spuriously reduced only for the highest spatial frequencies.

Fig. 6 shows, for four parvocellular neurons in LGN, how the different methods of analyzing responses affect the measured responsivity of cells. Recordings were made under the same conditions as those in which eye-movements were measured. Each panel shows two spatial-frequency tuning curves for a single neuron, derived from the same recorded impulse stream. Moving sinusoidal gratings of unit contrast were each presented 20 times in

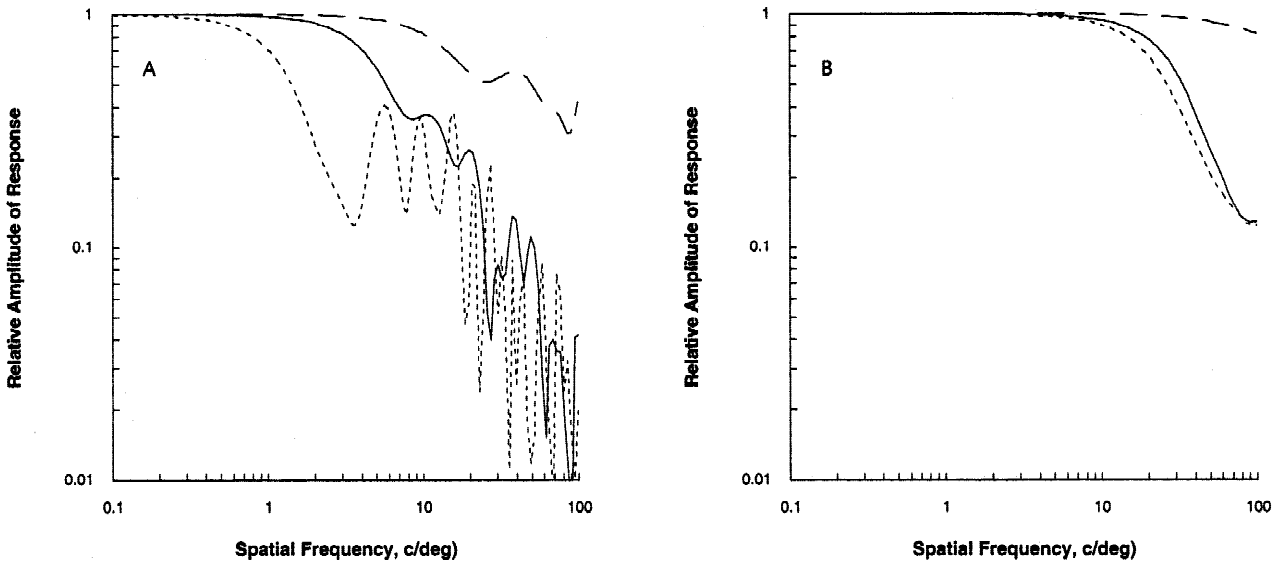

Fig. 5. Effect of eye-movements shown in Fig. 1 on responsivity of a simulated linear neuron to gratings of different spatial frequencies. Gratings of ten different spatial frequencies are each presented 20 times for 2 s, randomly interleaved. Movements of the eyes bring about changes in the relative phases of a particular grating on different trials. A: Relative response calculated for the case where we derive the amplitude from the averaged response accumulated in fixed phase (as in a poststimulus time histogram). Each line represents the effect of movements of one eye from Fig. 1 (long dashes, 1A,D; solid line, 1B,E; & short dashes, 1C,F). The neuron is assumed to be uniformly sensitive at all frequencies, so in the absence of eye-movements the lines would be flat with an amplitude of 1. B: Relative response calculated for the case where we derive separately the amplitude of each 2-s sample of response, then take the average of the set of amplitudes.

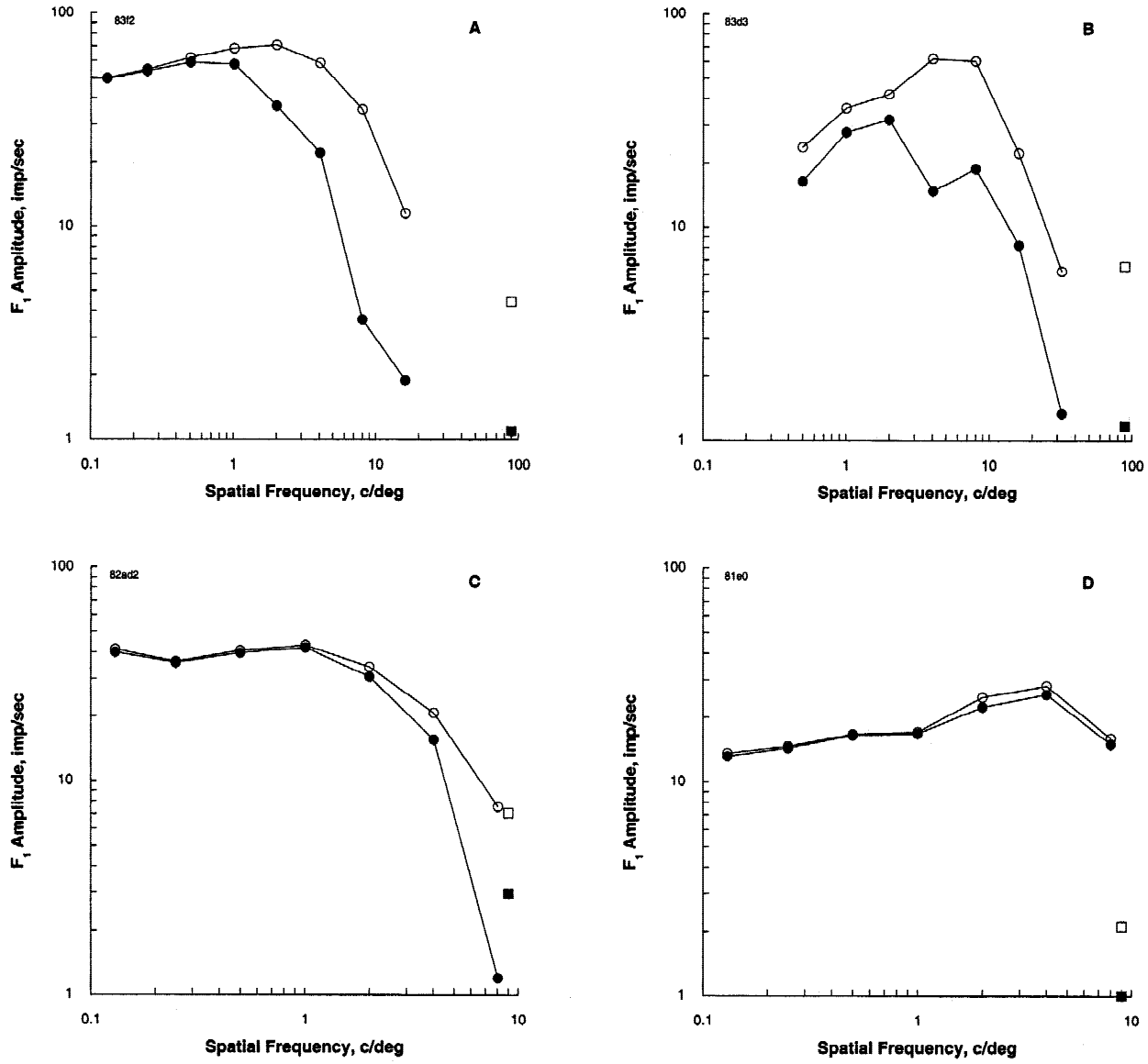

Fig. 6. Comparison of spatial-frequency tuning curves derived by two methods of analyzing impulse trains. Each panel shows, for a single neuron, the tuning curve derived from the amplitude of the first harmonic component of the average discharge (filled circles) and the average amplitude of the first harmonic component response, analyzed trail-by-trial (open circles). Squares show the corresponding measured amplitudes of the first harmonic in the maintained discharge in the absence of a grating. Data from neurons in Lankheet et al. (1998).

pseudorandom order, in trials lasting 2 s. Each trace shows the amplitude of the first harmonic component of the response, derived by taking the Fourier transform of the averaged response (filled circles) or by taking the average of the set of amplitudes measured on single trials (open circles). Among similar recordings from five monkeys, those analyzed in Figs. 6A and 6B (from the same monkey) represent the largest differences found; those in Figs. 6C and 6D (from two other monkeys) represent the smallest differences found. When applied to the maintained discharge in the absence of a grating (squares on the right), the trial-by-trial analysis recovers a larger amplitude of response than does the analysis of the average response, so we would expect the former method to yield a larger amplitude when the response is weak (rightmost points on the curves in Figs. 6B and 6C). That does not account for the progressive and large separation of the traces in Figs. 6A and 6B as spatial frequency is raised to that eliciting the largest response. In these cases, strong responses differ substantially in measured amplitude in a way that is most readily explained as the result of eye-movements. For neurons, such as simple cells in V1, that are sensitive to the spatial phase of the moving stimulus but have no spontaneous discharge, the average discharge rate, rather than the average amplitude of the fundamental Fourier component of discharge, will provide a reliable measure of response that is immune to the effects of eye-movements.

Eye-movements in paralyzed animals can lead to a profound underestimate of neuronal sensitivity to visual stimuli. Careful

choice of the method for analyzing responses can mitigate this, but even the most favorable methods will underestimate sensitivity to stimuli containing high spatial frequencies.

Acknowledgments

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