Synchronous Period-Doubling in Flicker Vision of Salamander and Man

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Crevier, Daniel W. and Markus Meister. Synchronous period-doubling in flicker vision of salamander and man. *J. Neurophysiol.* 79: 1869–1878, 1998. Periodic flashes of light have long served to probe the temporal properties of the visual system. Here we show that during rapid flicker of high contrast and intensity the eye reports to the brain only every other flash of light. In this regime, retinal ganglion cells of the salamander fire spikes on alternating flashes. Neurons across the entire retina are locked to the same flashes. The effect depends sharply on contrast and flash frequency. It results from a period-doubling bifurcation in retinal processing, and a simple model of nonlinear feedback reproduces the phenomenon. Pharmacological studies indicate that the critical feedback interactions require only cone photoreceptors and bipolar cells. Analogous period-doubling is observed in the human visual system. Under bright full-field flicker, the electroretinogram (ERG) shows a regime of period-doubling between 30 and 70 Hz. In visual evoked potentials from the occiput, the subharmonic component is even stronger. By analyzing the accompanying perceptual effects, we find that retinal period-doubling begins in the periphery of the visual field, and that it is the cause of a long mysterious illusory flicker pattern.

INTRODUCTION

A rapidly flashing light evokes the sensation of flicker, which eventually disappears as the flash frequency increases (Kelly 1972), a phenomenon known as flicker fusion. Our ability to perceive such flicker is limited in large part by temporal processing in the retina, and satisfying parallels have been established between human perception and the responses of retinal ganglion cells near the threshold of detection (Lee et al. 1989; Spekreijse et al. 1971; van de Grind et al. 1973). However, much of human vision involves stimuli far above the detection threshold, and strong flickering lights produce perceptual phenomena that are only poorly understood. For example, a large uniform flickering field evokes an impressive illusion of spatial patterns (Smythies 1959; Welpe 1970). Such flicker patterns have been known for centuries (Purkinje 1819), but have largely defied physiological explanation.

It is commonly held that the response of visual neurons repeats periodically at the frequency of the flash stimulus (Kelly 1972; van de Grind et al. 1973). Here we show that a bright large-field stimulus evokes dramatically different responses. Above a critical flash frequency, retinal ganglion cells systematically fire only on every other flash of light, ignoring the intervening flashes. The effect is found in both salamanders and humans and points to previously unknown aspects of retinal processing.

METHODS

Salamander eyecup recordings

The eyeball of a larval tiger salamander was hemisected, drained of vitreous, filled with Ringer medium (Meister et al. 1994), and placed in a well containing a reference electrode behind the sclera. Moist 95% O_2-5% CO_2 was blown over the preparation. Fiber signals from a sharp tungsten electrode inserted in the optic disk were filtered at 100–1,000 Hz, the ERG signal from a Ag/AgCl electrode in the eyecup was filtered at 1–1,000 Hz. A red light-emitting diode above the eyecup produced periodic square-wave flashes at frequency f. The mean intensity was constant in all reported experiments and equivalent to a flux of $7.7 \cdot 10^7$ photons/μm^2/s at 621 nm for the red cone receptors. Period-doubling occurred also at lower intensities, down to $4.9 \cdot 10^5$ photons/μm^2/s. Stimulus contrast, C, was measured as the intensity ratio (ON-OFF)/(ON + OFF). Animals were handled according to institutional guidelines.

Nonlinear feedback model

We analyzed a simple model of nonlinear feedback to account for period-doubling in the ERG response to periodic flashes (see Fig. 5*A*). Let x denote the amplitude of the response to a flash. Assume that $x = C \cdot g(y)$, where C is the stimulus contrast, and $g(y)$ is the response gain, which depends on the feedback variable y. Assume further that y increases by an amount $B \cdot x$ on a flash of amplitude x, and that y decreases continuously by exponential decay with time constant τ. In a sequence of flashes with frequency f, the response to the ith flash is therefore $x_i = C \cdot g(y_i)$ with

$$y_i = e^{-1/f\tau} B x_{i-1} + e^{-2/f\tau} B x_{i-2} + \cdots = e^{-1/f\tau}(B x_{i-1} + y_{i-1})$$
$$= e^{-1/f\tau}[BC \cdot g(y_{i-1}) + y_{i-1}]$$

Depending on the functional form of $g(y)$, this recurrence relation can become unstable leading to period-doubling and chaos. For the plots in Fig. 5, B and C, we chose $g(y) = 1/(1 + y^4)$. At each value of C and f, the recursion for y_i was iterated 200 times, and the subsequent 100 values of x_i were plotted along the ordinate. Note that the model has only two free parameters, B and τ, which set the scaling along the contrast and frequency axes.

Pharmacology

Drugs were added to Ringer medium, and the eyecup's contents were replaced several times to achieve the nominal concentrations at the retina. The pharmacological effects of all these agents have previously been analyzed in the retina of the salamander or closely related species, often using the eyecup preparation (Werblin 1991): 2-amino-4-phosphonobutyric acid (APB), 2-amino-7-phosphonoheptanoic acid (AP-7), D-aminovaleric acid (AVA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D-O-phosphoser-

ine (DOS), γ-aminobutyric acid (GABA), and *N*-methyl-D-aspartic acid (NMDA).

Human ERG

Subjects looked into a hemisected ping-pong ball, illuminated from behind by white light from a DC-operated tungsten source, which was modulated in square-wave fashion by a liquid crystal shutter (Stereographics). The average luminance was 5,000 cd/m^2, the ON/OFF intensity ratio was 100, and the rise and fall times of the intensity (measured between 0.1 and 0.9 of maximum) were <4 ms. No pupil dilation was used. The ERG was recorded with a bipolar Burian-Allen contact lens electrode and filtered at 1–1,000 Hz. For stimulation of central retina, the subject was moved back from the light source; for peripheral stimulation, black circles were glued to the hemisphere.

Human scalp potentials

The visual evoked potential (VEP) was recorded with the active electrode on the midline 5 cm above the inion, the reference electrode 8 cm anterior, and a ground electrode on the forehead. In some experiments, the reference electrode was 3 cm lateral of the active electrode, producing essentially identical results. Signals were filtered at 1–500 Hz. Stimulation was as described for ERG measurements, with one eye covered by a patch. All human subjects gave their informed consent.

RESULTS

Salamander retina

We first describe observations in the eyecup preparation of the tiger salamander. The retina was stimulated with bright periodic square-wave flashes. The collective response of ganglion cells was monitored with an extracellular tungsten electrode inserted into the optic disk, where the axons converge to form the optic nerve. The ERG was measured with an electrode in the vitreal medium.

Synchronous period-doubling

When the light flashed slowly, a volley of ganglion cell spikes was observed at the onset and two volleys at the offset of each flash (Fig. 1*A*). When the flash frequency increased above ~4 Hz, the ON volleys disappeared and a single OFF volley followed each flash (Fig. 1*B*). At flash frequencies >9 Hz, the ganglion cell response changed abruptly (Fig. 1*C*): now every other flash produced a volley of spikes, whereas the intervening flashes produced no response. We will call these the ''odd'' and ''even'' flashes, respectively. A parallel change occurred in the ERG: its response to the odd flashes was systematically larger than to the even flashes. Thus the response of retinal neurons was still periodic, but with a period twice that of the visual stimulus. When the flash rate was increased further, another change occurred above 12 Hz (Fig. 1*D*): the ganglion cells still responded to every other flash, but both the fiber volley and the ERG signal were larger for every fourth flash, so that the retinal response repeated only every 4 stimulus periods. Above 15 Hz, the response changed dramatically to a seemingly chaotic pattern, with no recognizable periodicity in the ERG signal or the fiber volleys (Fig. 1*E*).

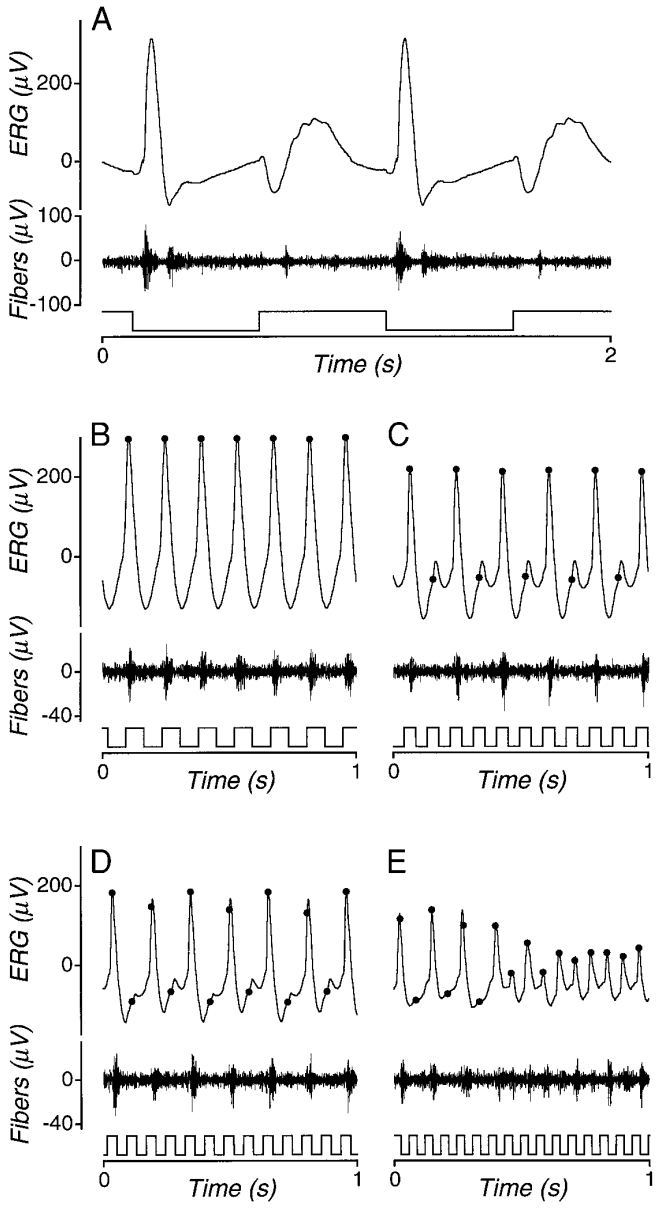

FIG. 1. Response of the salamander retina to uniform flicker. Recordings of the electroretinogram (ERG; *top trace*) and optic nerve fibers (*middle*) to the uniform flash stimulus (*bottom*), at a flash frequency of $f = 1$ Hz (*A*), 7 Hz (*B*), 11 Hz (*C*), 13 Hz (*D*), 16 Hz (*E*). Filled circles on the ERG trace indicate the value at a given delay during each flash interval, illustrating that the response repeats on every stimulus cycle in *B*, every 2 cycles in *C*, every 4 cycles in *D*, and lacks recognizable periodicity in *E*. For each flash rate, the delay was chosen to include the maximum of the waveform.

At sufficiently high flash rates, the ganglion cells appear to systematically ''ignore'' every other flash (Fig. 1*C*). This suggests some form of refractoriness within the network, by which the activation threshold is transiently elevated after a strong flash response. More strikingly, an entire population of nearby retinal ganglion cells acts in synchrony, responding to the same set of flashes, rather than choosing the odd or even flashes independently of each other. To assess the spatial extent of this synchrony, we recorded with two

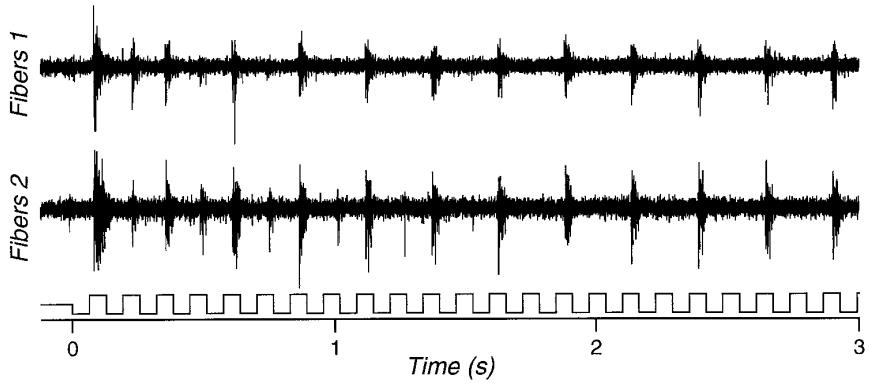

FIG. 2. Synchrony in alternating responses to uniform flicker. Fiber signals (*top 2 traces*) were recorded from 2 electrodes at opposite edges of the optic disk in the salamander eye cup. At *time 0*, the stimulus (*bottom trace*) changed from constant illumination to 8-Hz square-wave flicker of the same mean intensity.

extracellular electrodes from opposite margins of the optic disk, thus sampling two bundles of axons from separate regions of the retina. Figure 2 shows the time course of fiber volleys in the two regions following the sudden onset of the flashing stimulus. For a short time, every flash produced a burst of spikes, but within a few tenths of a second the volleys became restricted to alternating flashes. In this final state, bursts on the two electrodes were in phase, a result observed in all such two-electrode experiments. Thus the response synchrony extends across the entire retina.

The multiunit recordings from the optic disk cannot resolve the behavior of single neurons. To test whether individual ganglion cells respond systematically to every other flash, we isolated single-unit spikes recorded from cell bodies. Figure 3 shows the response of an OFF ganglion cell during a continuous frequency ramp. At low flash frequencies, the neuron fired after every flash. At a flash rate of $\sim$11 Hz, it suddenly switched to firing on alternating flashes. This occurred just after an alternating response appeared in the waveform of the ERG. Taken together with the above multiunit results, it appears that OFF cells across the entire retina systematically fire on the same set of alternating flashes.

How do different ganglion cells become synchronized? One might postulate that each individual neuron begins the alternating response rhythm in the same flash period, triggered by some change in the visual stimulus. The observations in Fig. 3 speak against this: where the alternating response begins, the flicker frequency changes very slowly, by $<$1% during each cycle. Thus the response properties of all ganglion cells in the retina would need to be identically calibrated to within 1% for the synchrony to arise independently. This is very unlikely. For example, different regions of the retina were illuminated with somewhat different intensity, due to the curvature of the eyecup, and intensity was found to significantly affect the threshold frequency for alternating responses (data not shown). Furthermore, on subsequent repeats of the same ramp stimulus, the alternating response initiated a few cycles earlier or later. In summary, the synchronization of many ganglion cells does not simply follow from their individual responses to the visual stimulus, but arises spontaneously within the retinal network, presumably mediated by lateral interactions. We will refer to this phenomenon as ''synchronous period-doubling.''

As a result of the retina-wide synchrony, period-doubling is easily observed in the ERG (Fig. 1*C* and Fig. 3). Figure 4*A* summarizes how the ERG response period depends on flash frequency with a ''bifurcation plot.'' As frequency increases, the abrupt branches in this plot indicate transitions from a period of 1 to 2, then 4 flash intervals. The subsequent smear along the ordinate reflects the chaotic response around 16 Hz. At higher flash frequencies the branches merge again, indicating successive halving of the response period, until, above 30 Hz, the ERG signal was again periodic with the stimulus. These changes in the response period occurred very suddenly, within a fraction of 1 Hz. In other experiments we varied the contrast of the flashes, while keeping the flash frequency constant (Fig. 4*B*). At low contrast, the ERG followed the stimulus, but its period abruptly switched to 2 and then 4 flash intervals as the contrast increased. At the highest contrasts, the response again became chaotic. Note that the peak amplitude of the ERG grew linearly with contrast over most of this range, suggesting that the signaling processes involved were not saturated by the visual stimulus.

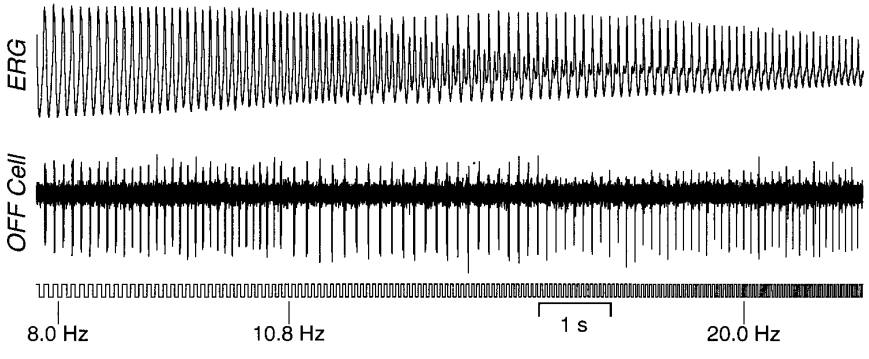

FIG. 3. Period-doubling during a continuous frequency ramp. Recordings of the salamander ERG (*top trace*) and extracellular spikes from a single OFF cell (*middle trace*) in response to square-wave flicker (*bottom trace*) that increased smoothly in frequency.

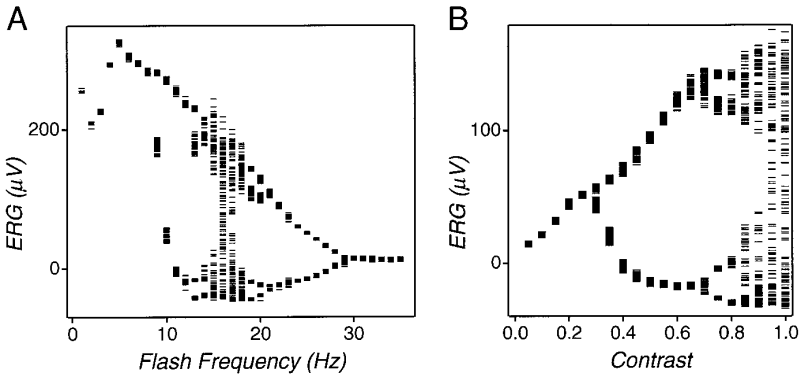

FIG. 4. Bifurcation plot of the salamander ERG. *A*: ERG amplitude as a function of flash frequency at contrast $C = 1.0$. For each frequency value on the abscissa, the ERG was recorded for 12 s. The maximum of this waveform was located, and the values at the corresponding phase in all other flash intervals were plotted on the ordinate. These correspond to the markers on the ERG trace in Fig. 1, *B–E*. *B*: ERG amplitude as a function of contrast at flash frequency $f = 16$ Hz, displayed as in *A*.

Nonlinear dynamics

This sequence of successive period-doublings has close parallels in the nonlinear dynamics of other physical and mathematical systems (Feigenbaum 1983; Rasband 1990). Often, an accelerating sequence of period-doublings leads to a chaotic regime (Canavier et al. 1990; Guevara et al. 1981). Many nonlinear systems that exhibit period-doubling bifurcations contain some form of negative feedback by which a strong response during one cycle of the input reduces the response to the subsequent cycle. Indeed, a simple model of nonlinear feedback (Fig. 5*A* and METHODS) reproduces the phenomenology observed on the salamander ERG. Here, the peak amplitude of the ERG, x, is taken to be proportional to the amplitude of the light flash, C, and a gain factor, $g(y)$. This gain, in turn, depends on the amplitude of recent flash responses through the feedback variable y. Figure 5, *B* and *C*, shows the behavior of this mechanism as a function of flash frequency and contrast. The model predicts a sequence of period-doublings as the frequency is increased, followed by a reverse sequence of period-halvings until a period of one is reached again at the highest flash rates. Similarly, increasing the contrast leads to a series of period-doublings ending in chaos. With just two parameters, the model can match the approximate locations of the branch points in the experimentally observed sequence (Fig. 4, *A* and *B*). Moreover, it also matches the decrease in ERG amplitude with increasing flash rate (Fig. 4*A*). Thus it is plausible that period-doubling in retinal responses results from a nonlinear gain control.

Circuit mechanisms

To identify the mechanisms that might produce such effects, we restricted the active circuitry pharmacologically, with a particular aim at negative feedback elements. The phototransduction cascade in rod and cone receptors includes various feedback loops that serve to terminate the light response and adjust its gain to the mean intensity (Baylor 1996). To isolate the photoreceptors from the rest of the retina, we blocked their glutamatergic transmission to second-order cells, by adding to the medium 100 μM APB (Nawy and Jahr 1990) (see METHODS for full names of all compounds) and 50 μM CNQX (Hensley et al. 1993). The ERG derived from photoreceptors alone was strictly periodic with the stimulus at all flash frequencies (Fig. 6*B*) and showed no indication of the period-doublings observed under control conditions (Fig. 6*A*). The same result was obtained when photoreceptors were isolated using 100 mM aspartate (Shimazaki et al. 1984), or 100 μM APB with 5 mM kynurenic acid (Xu et al. 1991). Clearly the nonlinearities of phototransduction are not responsible for period-doubling.

To test the role of refractoriness in ganglion cells, we silenced their action potentials (5 μM tetrodotoxin): the ERG signal still showed frequency-dependent period-dou-

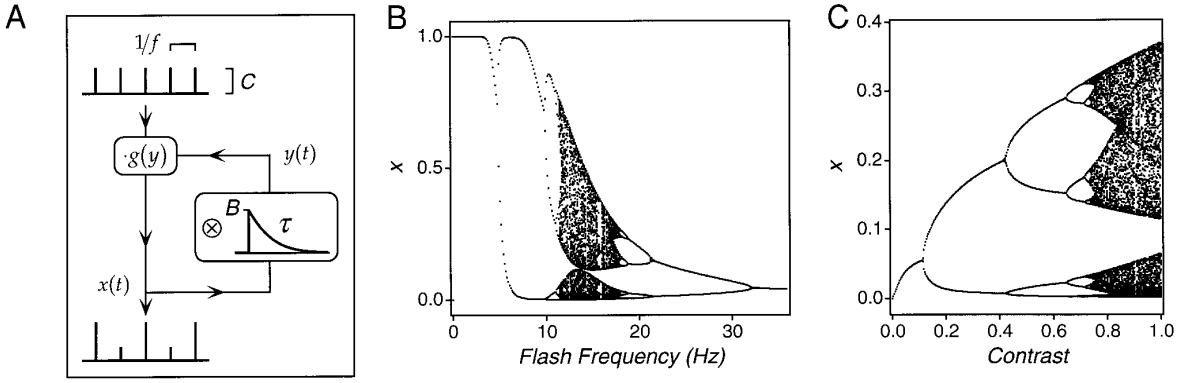

FIG. 5. Period-doubling in a model of nonlinear feedback. *A*: diagram of the model: input pulses are multiplied by a variable gain that depends on the amplitude of preceding output pulses. See text for details. *B* and *C*: bifurcation plots of the output pulse amplitude, x, as a function of flash frequency (*B*) and contrast (*C*). $B = 35$, $\tau = 58$ ms.

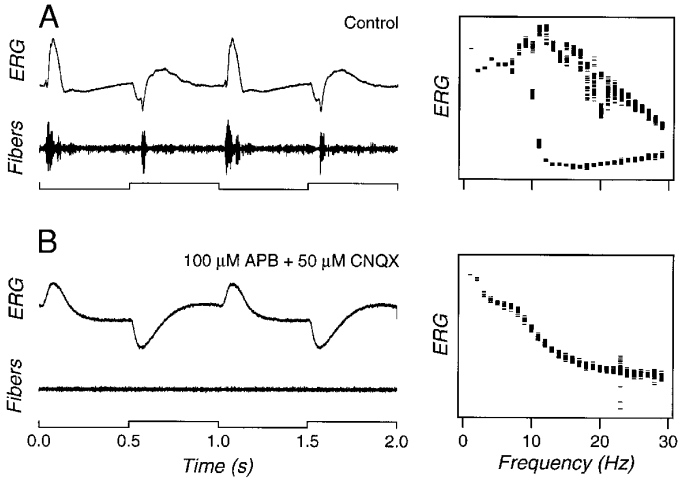

FIG. 6. Phototransduction currents do not undergo period-doubling. *A*: control recordings from the salamander eye cup in Ringer medium. *Left*: ERG (*top trace*) and optic nerve fiber signals (*middle trace*) responding to a 1-Hz flash (*bottom trace*). *Right*: bifurcation plot of ERG amplitude vs. flash frequency, displayed as in Fig. 4*A*. *B*: repeat measurements after the photoreceptor ERG was isolated by adding to the medium 100 μM 2-amino-4-phosphonobutyric acid (APB) and 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).

bling. In 1 mM NMDA, which strongly polarizes and thus inactivates ganglion cells and most amacrine cells (Slaughter and Miller 1983), period-doubling still persisted. Thus the effect likely does not require circuitry in the inner retina. To test for destructive interference between responses to the onset and offset of the light flashes, we blocked the ON pathway at the photoreceptor synapse (100 μM APB). As expected, ON-ganglion cell responses disappeared, but the remaining optic disk signals and the ERG still showed strong period-doubling. On the other hand, no period-doubling occurred when ionotropic glutamate receptors were blocked, reducing the functional circuit to cones and ON bipolars [5 mM kynurenic acid; or 50 μM CNQX with 100 μM AP-7 (Diamond and Copenhagen 1995)]. Blocking the light response of horizontal cells [5 mM DOS (Slaughter and Miller 1985)], which eliminates their negative feedback onto cone terminals, had no effect on period-doubling. Finally, we interfered with other negative feedback pathways in the retina using a cocktail of blockers for the inhibitory transmitters GABA and glycine [250 μM picrotoxin (Maguire et al. 1989), 100 μM strychnine (Belgum et al. 1984), 2 mM AVA (Hare and Owen 1996), and 1 mM phaclofen]. As expected, this produced a large increase in ganglion cell firing activity (Fig. 7). It also eliminated the oscillatory potentials in the ON-response of the ERG, thought to derive from inhibitory amacrine circuits (Hamasaki et al. 1990; Wachtmeister and Dowling 1978). However, the ERG still underwent period-doubling during frequency ramps.

Thus synchronous period-doubling originates after the photoreceptors but before ganglion cells. It occurs in the isolated OFF pathway, consistent with the fact that ON responses are lost at lower flash frequencies. Period-doubling does not seem to rely on intercellular inhibitory feedback. The minimal circuit required to produce period-doubling under all the above conditions consists of only cones and OFF-bipolar cells.

Human vision

ERG. To explore whether period-doubling occurs in human vision, we measured the ERG of three subjects under bright full-field periodic flashes. Figure 8*A* illustrates the ERG waveform at two flash frequencies. At 26 Hz the ERG response repeated identically with every flash, but at 46 Hz alternating flashes produced large or small peaks. This alternating rhythm was maintained without breaks throughout a 200-s recording. Note the close analogy to ERG waveforms from the salamander eyecup (Fig. 1, *B* and *C*).

The strength of period-doubling in these signals is revealed by their power spectrum (Fig. 8*B*). Under 26-Hz stimulation the spectrum contains peaks only at the stimulus frequency (f) and its higher harmonics ($2f, 3f, \cdots$). However, under 46-Hz stimulation, one also finds peaks at the even subharmonics of the stimulus frequency ($f/2$, $f/4$) or their multiples ($3f/2$, $3f/4$, $5f/4$). This occurs because certain components of the response repeat only over even multiples of the stimulus period. One obtains a simple measure of this period-doubling by comparing the power at f with that at the subharmonics (Fig. 8*C*): period-doubling was strictly limited to the range between 30 and 70 Hz. At both ends of the range, the effect disappeared very suddenly: the relative power of the subharmonics changed by a factor of 100 over 10 Hz, similar to the sharp frequency dependence seen in salamander (Figs. 3 and 4*A*). The absolute frequencies at which alternating responses were observed are about threefold higher in humans than in the salamander. This correlates well with the relative speeds of other retinal processes; for example, the flash response of primate cones (Schnapf et al. 1990) is two- to threefold faster than that of salamander cones (Matthews et al. 1990).

VISUAL EVOKED POTENTIALS. Whereas the flash ERG is dominated by contributions from the outer retina, the time structure of visual signals that reach the brain is revealed in

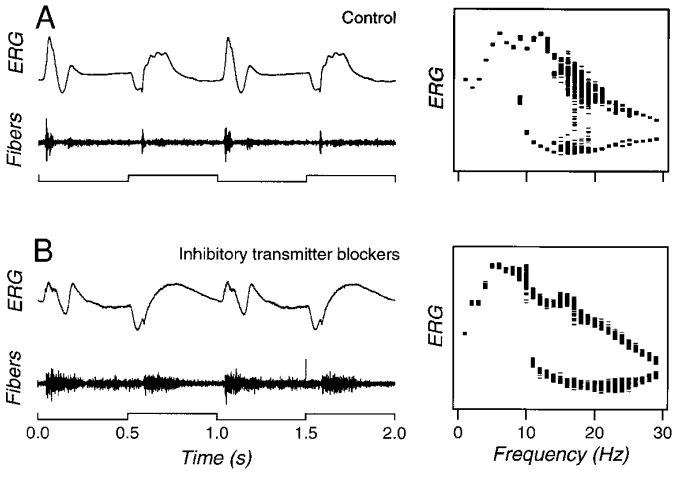

FIG. 7. Inhibitory synaptic transmission is not required for period-doubling. *A*: control recordings from the salamander eye cup in Ringer medium. *Left*: ERG (*top trace*) and optic nerve fiber signals (*middle trace*) responding to a 1-Hz flash (*bottom trace*). *Right*: bifurcation plot of ERG amplitude vs. flash frequency, displayed as in Fig. 4*A*. *B*: repeat measurements after adding to the medium blockers of inhibitory transmission: 250 μM picrotoxin, 100 μM strychnine, 2 mM D-aminovaleric acid (AVA), 1 mM phaclofen.

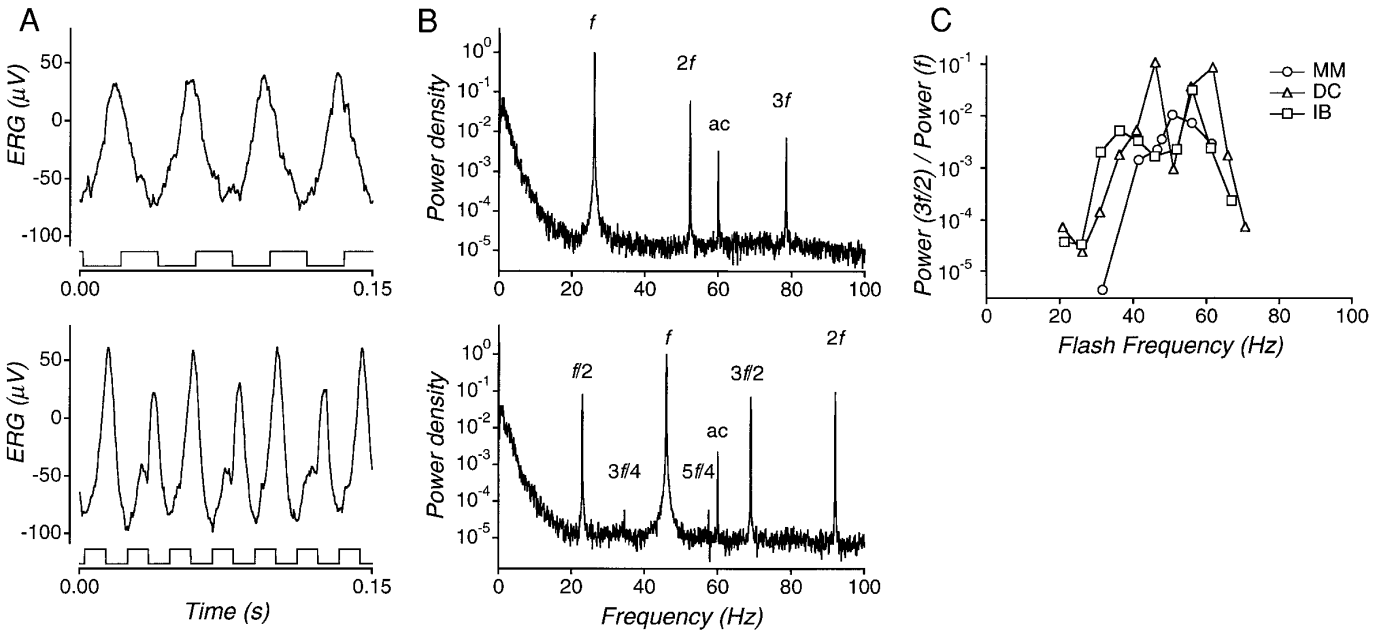

FIG. 8. Response of the human ERG to uniform flicker. *A*: raw waveform recorded at flash frequencies of $f = 26$ Hz (*top*) and 46 Hz (*bottom*). *B*: power spectrum of the ERG at $f = 26$ Hz (*top*) and 46 Hz (*bottom*), computed over a 200-s window, and normalized to the power density at f. Peaks at harmonics and subharmonics of the flash frequency are labeled; ac, 60-Hz line interference. *C*: relative strength of period-doubling in the ERG of 3 subjects, measured as a function of flash frequency, by the ratio of (power at $3f/2$) to (power at f). The subharmonic modulation was evaluated at $3f/2$ rather than $f/2$ because of the severe increase in the background power at low frequencies (see *B*), which is mostly due to eye movement transients.

scalp potentials from the occipital part of the head. This VEP to a periodic stimulus is generally thought to vary at the stimulus frequency and its higher harmonics (Regan 1989). Under the above stimulus conditions we observed very different behavior (Fig. 9): At $f = 51$ Hz, the VEP had a period of two flash intervals, and the dominant component of the power spectrum was at $f/2$. At 16 Hz, there was no indication of period-doubling in the VEP waveform or its power spectrum. Generally, the degree of period-doubling, as measured by the power in subharmonics of the flash frequency, was much greater in the VEP response than in the ERG (compare Figs. 9*C* and 8*C*). This can be understood because the ERG includes signals from the outer retina that still follow every flash (see Figs. 1*C* and 6*B*). Near $f = 50$ Hz, the VEP power at the $f/2$ subharmonic even exceeded the power at the stimulus frequency, f. In this regime, it appears that the majority of retinal ganglion cells respond exclusively to every other flash, and do so in synchrony across the visual field. This affects visual processing in all subsequent visual circuits.

PERCEPTION. All human subjects reported strong perceptual effects during these experiments. At flash frequencies near 50 Hz, there was little or no perceptible flicker, but the field showed a strong spatial pattern: a distinct yellow region in the center of gaze, 35–50° diam, surrounded by an intensely bright, blue-white region in the periphery. Note that this illusion is a striking violation of the Talbot-Plateau law, which states that for flicker frequencies above perceptual fusion the field should have the same appearance as a steady light of the same mean intensity (van de Grind et al. 1973).

This yellow spot was first described by Welpe (Welpe 1970). It is of retinal origin, because binocular stimulation produced two yellow spots of slightly different shape, alternating in binocular rivalry. We found that the spot's diameter increased at both lower and higher flash rates. Because the strength of the $f/2$ signal in the ERG decreases on either side of 50 Hz (Fig. 7*C*), one suspects that period-doubling originates in the peripheral region outside the yellow spot, possibly because peripheral retina is more sensitive at high flicker rates (Seiple and Holopigian 1996). This was confirmed by varying the visual display: limiting the flash stimulus to the central 65° abolished the $f/2$ components in the ERG, whereas occluding the central 35° of the flashing field had no such effect.

These observations suggest that near $f = 50$ Hz the ganglion cells in the periphery respond synchronously at $f/2$, whereas those in the center respond at f or have lost any phase-locking to the flashes. The resulting difference in spike patterns received from central and peripheral neurons may evoke the marked increase of perceived brightness in the periphery.

DISCUSSION

Our view of temporal processing in the visual system is revised in several aspects. From previous work, it had been assumed that the response of retinal neurons degrades gracefully at high temporal frequencies, with a gradual loss of phase-locking to the stimulus (Enroth 1952; van de Grind et al. 1973). Instead, under certain stimulus conditions, the

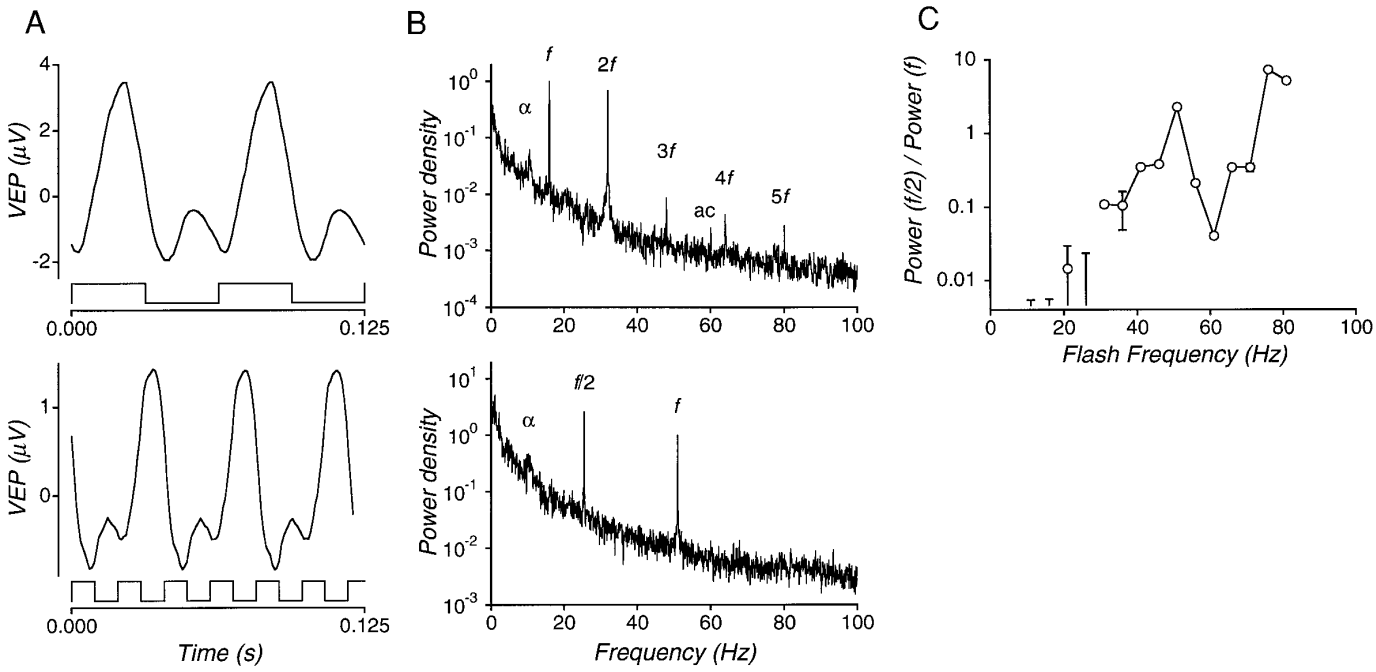

FIG. 9. Response of the human visual evoked potential (VEP) to uniform flicker. *A*: average VEP waveform of *subject MM,* triggered on the odd flashes over a 100-s recording, at $f = 16$ Hz (*top*) and 51 Hz (*bottom*). *B*: power spectrum of the VEP at $f = 16$ Hz (*top*) and 51 Hz (*bottom*). Peaks at harmonics and subharmonics of the flash frequency are labeled; ac, 60-Hz line interference; α, alpha waves. *C*: relative strength of period-doubling in the VEP, measured as a function of flash frequency, by the ratio of (power at $f/2$) to (power at f). Error bars indicate uncertainty due to the background electroencephalogram (EEG) power. For flicker at $f \leq 26$ Hz, the power spectrum had no significant peak at $f/2$.

retinal output undergoes a series of successive period-doublings before flicker fusion is reached. In this regime, retinal responses are synchronized across the retina, over distances of several centimeters in the human eye. Underlying this, there appears to be a mechanism of strong nonlinear feedback, possibly in retinal bipolar cells, along with lateral coupling circuits that promote the global synchrony. The resulting temporal and spatial structure of the optic nerve signals affects all subsequent visual processing and leads to illusory percepts under high-frequency flicker.

History

There have been isolated reports of subharmonic responses to a periodic flicker stimulus. Remarkably, they were seen in some of the earliest recordings from retina (Adrian and Matthews 1928). In optic nerve signals from the eel eye, period-doubling occurred at ~14 Hz, similar to the first bifurcation frequency we measured in salamander (Fig. 4*A*). Later on, Best and Bohnen (1957) reported ''alternating potentials'' in the human ERG under bright square-wave flicker. This subharmonic response was evident at frequencies between 40 and 60 Hz, similar to the range reported here (Fig. 7*C*). In visual evoked potentials, subharmonic components were thought to be rare (Regan 1972), but exceptions have been reported, notably in dog (Lopes da Silva et al. 1970) and fish (Karamursel and Bullock 1994). None of these observations were pursued to trace their cellular origins or implications for visual function.

By contrast, this subject has received considerable attention in the auditory system. For sound frequencies above ~100 Hz, auditory nerve neurons no longer fire in every cycle of the pressure wave. Yet their patterns of firing somehow encode both frequency and intensity of the sound. An early ''volley theory'' proclaimed that individual nerve fibers fire systematically on every nth cycle of the sound wave, and that nerve fibers from neighboring hair cells are locked to different cycles (Wever 1949). In this way the collection of auditory afferents would faithfully produce one spike volley for every cycle. This idea has been thoroughly disproved. Individual auditory nerve fibers fire stochastically in each cycle. As a result, the histogram of interspike intervals in such a spike train shows all multiples of the stimulus period, with probabilities declining roughly exponentially with interval length (Kiang 1965). Power spectra of the spike trains in this regime show no indication of a subharmonic component at $1/n$th of the stimulus frequency (Javel et al. 1988). Finally, nearby auditory fibers are statistically independent in whether they fire during the same cycle or not (Kiang 1990). On all counts, this behavior at high stimulus frequencies is very different from the period-doubling we describe. Thus period-doubling is not a necessary consequence of high-frequency stimulation, but arises from a specific type of processing within the retinal network.

Mechanisms

Our pharmacological analysis showed that the photoreceptors themselves do not produce period-doubling in the ERG. Perturbations of neurons in the inner retina did not abolish

the effect. Also, inhibitory feedback among neurons was not required. It appears that period-doubling arises at the synapse between photoreceptors and bipolar cells. Several candidate mechanisms exist, although we have no evidence yet to distinguish them.

For example, the nonlinear feedback might involve a delayed voltage-activated conductance in the bipolar cell membrane (Klumpp et al. 1995; Tessier-Lavigne et al. 1988) that reduces the gain of the light response for a short period after a strong flash (Lasansky 1992; Mao et al. 1998). In this context, the model of Fig. 5*A* might have the following components: synaptic input current during the first flash cycle depolarizes the bipolar cell membrane potential (x), which leads to a delayed activation (with time constant τ) of an outward conductance (y). This reduces the membrane impedance (g), which, in turn, limits the cell's response to synaptic current from the subsequent flash. The synchronization of nearby bipolar cells could be achieved if they are electrically coupled (Cohen and Sterling 1990; Hare and Owen 1990; Raviola and Gilula 1975; Saito and Kujiraoka 1988). For two bipolar cells that respond to alternating flashes in the same phase, electrical coupling will have no effect, because they produce the same membrane potential at all times. However, if they respond out of phase, electrical coupling reduces the swing of the membrane potential in each cell, thus reducing the amount of negative feedback. Therefore the threshold for period-doubling of the synchronous mode is lower than for the asynchronous mode, and synchrony will be favored as period-doubling develops.

A similar mechanism might operate presynaptically: the membrane of the photoreceptor inner segment contains an inward-rectifying conductance, I_h, activated by hyperpolarization below −50 mV, and with a reversal potential above the cell's resting potential (Bader and Bertrand 1984). In response to a strong flash of light, the outer segment current shuts off, the inner segment rapidly hyperpolarizes, but after a short delay I_h is activated and repolarizes the cell to a plateau (Baylor et al. 1984). While this conductance is active, the subsequent flash will produce a smaller voltage response. In lizard cones, the time constant for activation of I_h has been measured near 52 ms (Maricq and Korenbrot 1990), comparable with the value of $\tau = 58$ ms derived from Fig. 5, *B* and *C*. This feedback loop could lead to period-doubling in the membrane voltage at the cone terminal, and thus in the response of second-order neurons. On the other hand, the conductance changes at the inner segment produce no noticeable change in the circulating current through the outer segment membrane (Baylor et al. 1984), which makes the photoreceptor's contribution to the ERG. This could explain why the isolated photoreceptor ERG never showed an alternating response (Fig. 6*B*). In this scheme, photoreceptors could become synchronized if they are electrically coupled near their terminals (Attwell et al. 1984; Schneeweis and Schnapf 1995; Tsukamoto et al. 1992), for the same reasons invoked above for bipolar cells.

Clearly, the above proposals are speculative, and intracellular recordings would help pinpoint the site of period-doubling. We attempted to interfere with lateral coupling by treating the salamander retina with putative gap-junction blockers, such as heptanol or intracellular acidification by acetate (DeVries and Schwartz 1989; Spray and Burt 1990). Unfortunately, these treatments have rather nonspecific effects throughout the retina, and light responses often changed substantially or ceased before period-doubling was affected. More specific blockers will be needed before the role of gap-junctions can be tested directly.

Visual processing

The mechanisms discussed above act to reduce the gain of the photoreceptor or the bipolar cell in the face of strong swings of the light intensity. This would help stabilize the neuron's response and keep the membrane potential in a range where the synaptic output is still modulated. Such a feedback pathway may well underlie the rapid contrast gain control documented in cat retina (Victor 1987).

More generally, one expects that such a gain control would serve any neuron in dealing with strong fluctuations of its input signals. In fact, we have some indications that period-doubling also occurs beyond the retina. For example, the alternating response in the human ERG was abolished when the stimulus covered only the center 65°, whereas the VEP still showed a strong subharmonic component under these conditions. Similarly, reducing the light intensity by a factor of 4 abolished period-doubling in the ERG, but not in the VEP (data not shown). This suggests that period-doubling can arise at a second site, possibly in cortical circuits.

At flash frequencies of ~10–30 Hz, we observed no subharmonics in the ERG or the VEP, but human subjects reported dramatic visual illusions: the field broke up into varying geometric patterns that appeared to flicker violently, with neighboring regions flashing in counterphase. The phenomenology of these flicker patterns has been described extensively (Purkinje 1819; Smythies 1959). They could be explained if neurons in a retinotopic map, for example in visual cortex, respond at $f/2$, but their activity is not globally synchronized. If two adjacent regions respond to the odd and even flashes, respectively, the percept of spatial structure with counterphase flicker could arise. The shape of the regions corresponding to the two phases would reflect the circuits of lateral inhibition and excitation within the map. Because adjacent out-of-phase regions make opposite contributions to large-scale field potentials, one would not observe such local period-doubling in the VEP, but it could well be studied with single-unit electrodes.

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REFERENCES

ADRIAN, E. D. AND MATTHEWS, R. The action of light on the eye. Part III. The interaction of retinal neurones. *J. Physiol. (Lond.)* 65: 273–298, 1928.

ATTWELL, D., WILSON, M., AND WU, S. M. A quantitative analysis of interactions between photoreceptors in the salamander (*Ambystoma*) retina. *J. Physiol. (Lond.)* 352: 703–737, 1984.

BADER, C. R. AND BERTRAND, D. Effect of changes in intra- and extracellular sodium on the inward (anomalous) rectification in salamander photoreceptors. *J. Physiol. (Lond.)* 347: 611–631, 1984.

BAYLOR, D. How photons start vision. *Proc. Natl. Acad. Sci. USA* 93: 560–565, 1996.

BAYLOR, D. A., MATTHEWS, G., AND NUNN, B. J. Location and function of voltage-sensitive conductances in retinal rods of the salamander, *Ambystoma tigrinum*. *J. Physiol. (Lond.)* 354: 203–223, 1984.

BELGUM, J. H., DVORAK, D. R., AND MCREYNOLDS, J. S. Strychnine blocks transient but not sustained inhibition in mudpuppy retinal ganglion cells. *J. Physiol. (Lond.)* 354: 273–286, 1984.

BEST, W. AND BOHNEN, K. Alternierende Potentiale im Elektroretinogramm des Menschen bei Flimmerbelichtung. *Acta Ophthalmol.* 35: 273–278, 1957.

CANAVIER, C. C., CLARK, J. W., AND BYRNE, J. H. Routes to chaos in a model of a bursting neuron. *Biophys. J.* 57: 1245–1251, 1990.

COHEN, E. AND STERLING, P. Demonstration of cell types among cone bipolar neurons of cat retina. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 330: 305–321, 1990.

DEVRIES, S. H. AND SCHWARTZ, E. A. Modulation of an electrical synapse between solitary pairs of catfish horizontal cells by dopamine and second messengers. *J. Physiol. (Lond.)* 414: 351–375, 1989.

DIAMOND, J. S. AND COPENHAGEN, D. R. The relationship between light-evoked synaptic excitation and spiking behaviour of salamander retinal ganglion cells. *J. Physiol. (Lond.)* 487: 711–725, 1995.

ENROTH, C. The mechanism of flicker and fusion studied in single retinal elements in the dark-adapted eye of the cat. *Acta Physiol. Scand.* 27, *Suppl.* 100: 1–67, 1952.

FEIGENBAUM, M. Universal behavior in nonlinear systems. *Physica* 7D: 16–39, 1983.

GUEVARA, M. R., GLASS, L., AND SHRIER, A. Phase locking, period-doubling bifurcations, and irregular dynamics in periodically stimulated cardiac cells. *Science* 214: 1350–1353, 1981.

HAMASAKI, D. I., TUCKER, G. S., AND MAGUIRE, G. W. Alterations of the cat's electroretinogram induced by the lesioning of the indoleamine-accumulating amacrine cells. *Ophthalmic Res.* 22: 19–30, 1990.

HARE, W. A. AND OWEN, W. G. Spatial organization of the bipolar cell's receptive field in the retina of the tiger salamander. *J. Physiol. (Lond.)* 421: 223–245, 1990.

HARE, W. A. AND OWEN, W. G. Receptive field of the retinal bipolar cell—a pharmacological study in the tiger salamander. *J. Neurophysiol.* 76: 2005–2019, 1996.

HENSLEY, S. H., YANG, X. L., AND WU, S. M. Identification of glutamate receptor subtypes mediating inputs to bipolar cells and ganglion cells in the tiger salamander retina. *J. Neurophysiol.* 69: 2099–2107, 1993.

JAVEL, E., MCGEE, J. A., HORST, J. W., AND FARLEY, G. R. Temporal mechanisms in auditory stimulus coding. In: *Auditory Function: Neurobiological Bases of Hearing*, edited by G. M. Edelman, W. E. Gall, and W. M. Cowan. New York: Wiley, 1988, p. 515–558.

KARAMURSEL, S. AND BULLOCK, T. H. Dynamics of event-related potentials to trains of light and dark flashes: responses to missing and extra stimuli in elasmobranch fish. *Electroencephalogr. Clin. Neurophysiol.* 90: 461–471, 1994.

KELLY, D. H. Flicker. In: *Handbook of Sensory Physiology*, edited by D. Jameson and L. M. Hurvich. New York: Springer, 1972, p. 273–302.

KIANG, N.Y.S. *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve*. Cambridge, MA: MIT Press, 1965.

KIANG, N.Y.S. Curious oddments of auditory-nerve studies. *Hear. Res.* 49: 1–16, 1990.

KLUMPP, D. J., SONG, E. J., ITO, S., SHENG, M. H., JAN, L. Y., AND PINTO, L. H. The Shaker-like potassium channels of the mouse rod bipolar cell and their contributions to the membrane current. *J. Neurosci.* 15: 5004–5013, 1995.

LASANSKY, A. Properties of depolarizing bipolar cell responses to central illumination in salamander retinal slices. *Brain Res.* 576: 181–196, 1992.

LEE, B. B., MARTIN, P. R., AND VALBERG, A. Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. *J. Physiol. (Lond.)* 414: 223–243, 1989.

LOPES DA SILVA, F. H., ROTTERDAM, A. V., STORM VAN LEEUWEN, W., AND TIELEN, A. M. Dynamic characteristics of visual evoked potentials in the dog. I. Cortical and subcortical potentials evoked by sine wave modulated light. *Electroencephalogr. Clin. Neurophysiol.* 29: 246–259, 1970.

MAGUIRE, G., LUKASIEWICZ, P., AND WERBLIN, F. Amacrine cell interactions underlying the response to change in the tiger salamander retina. *J. Neurosci.* 9: 726–735, 1989.

MAO, B. Q., MACLEISH, P. R., AND VICTOR, J. D. The intrinsic dynamics of retinal bipolar cells isolated from tiger salamander. *Vis. Neurosci.* In press.

MARICQ, A. V. AND KORENBROT, J. I. Inward rectification in the inner segment of single retinal cone photoreceptors. *J. Neurophysiol.* 64: 1917–1928, 1990.

MATTHEWS, H. R., FAIN, G. L., MURPHY, R. L., AND LAMB, T. D. Light adaptation in cone photoreceptors of the salamander: a role for cytoplasmic calcium. *J. Physiol. (Lond.)* 420: 447–469, 1990.

MEISTER, M., PINE, J., AND BAYLOR, D. A. Multi-neuronal signals from the retina: acquisition and analysis. *J. Neurosci. Methods* 51: 95–106, 1994.

NAWY, S. AND JAHR, C. E. Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. *Nature* 346: 269–271, 1990.

PURKINJE, J. E. *Beiträge zur Kenntniss des Sehens in subjectiver Hinsicht.* Prague: J. G. Calve, 1819.

RASBAND, S. N. *Chaotic Dynamics of Nonlinear Systems*. New York: Wiley, 1990.

RAVIOLA, E. AND GILULA, N. B. Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. A freeze-fracture study in monkeys and rabbits. *J. Cell Biol.* 65: 192–222, 1975.

REGAN, D. *Evoked Potentials in Psychology, Sensory Physiology and Clinical Medicine*. London: Chapman & Hall, 1972.

REGAN, D. *Human Brain Electrophysiology: Evoked Potentials and Evoked Magnetic Fields in Science and Medicine*. New York: Elsevier, 1989.

SAITO, T. AND KUJIRAOKA, T. Characteristics of bipolar-bipolar coupling in the carp retina. *J. Gen. Physiol.* 91: 275–287, 1988.

SCHNAPF, J. L., NUNN, B. J., MEISTER, M., AND BAYLOR, D. A. Visual transduction in cones of the monkey *Macaca fascicularis*. *J. Physiol. (Lond.)* 427: 681–713, 1990.

SCHNEEWEIS, D. M. AND SCHNAPF, J. L. Photovoltage of rods and cones in the macaque retina. *Science* 268: 1053–1056, 1995.

SEIPLE, W. AND HOLOPIGIAN, K. Outer-retina locus of increased flicker sensitivity of the peripheral retina. *J. Opt. Soc. Am. A* 13: 658–666, 1996.

SHIMAZAKI, H., KARWOSKI, C. J., AND PROENZA, L. M. Aspartate-induced dissociation of proximal from distal retinal activity in the mudpuppy. *Vision Res.* 24: 587–595, 1984.

SLAUGHTER, M. M. AND MILLER, R. F. The role of excitatory amino acid transmitters in the mudpuppy retina: an analysis with kainic acid and *N*-methyl aspartate. *J. Neurosci.* 3: 1701–1711, 1983.

SLAUGHTER, M. M. AND MILLER, R. F. Identification of a distinct synaptic glutamate receptor on horizontal cells in mudpuppy retina. *Nature* 314: 96–97, 1985.

SMYTHIES, J. R. The stroboscopic patterns. II. The phenomenology of the bright phase and after-images. *Br. J. Psychol.* 50: 305–324, 1959.

SPEKREIJSE, H., VAN NORREN, D., AND VAN DEN BERG, T. J. Flicker responses in monkey lateral geniculate nucleus and human perception of flicker. *Proc. Natl. Acad. Sci. USA* 68: 2802–2805, 1971.

SPRAY, D. C. AND BURT, J. M. Structure-activity relations of the cardiac gap junction channel. *Am. J. Physiol.* 258 (*Cell Physiol.* 27): C195–C205, 1990.

TESSIER-LAVIGNE, M., ATTWELL, D., MOBBS, P., AND WILSON, M. Membrane currents in retinal bipolar cells of the axolotl. *J. Gen. Physiol.* 91: 49–72, 1988.

TSUKAMOTO, Y., MASARACHIA, P., SCHEIN, S. J., AND STERLING, P. Gap junctions between the pedicles of macaque foveal cones. *Vision Res.* 32: 1809–1815, 1992.

VAN DE GRIND, W. A., GRÜSSER, O.-J., AND LUNKENHEIMER, H.-U. Temporal transfer properties of the afferent visual system. In: *Handbook of Sensory Physiology*, edited by R. Jung. New York: Springer-Verlag, 1973, p. 431–573.

VICTOR, J. D. The dynamics of the cat retinal X cell centre. *J. Physiol. (Lond.)* 386: 219–246, 1987.

WACHTMEISTER, L. AND DOWLING, J. E. The oscillatory potentials of the mudpuppy retina. *Invest. Ophthalmol. Vis. Sci.* 17: 1176–1188, 1978.

WELPE, E. Über die Strukturierung des Gesichtsfeldes bei intermittierender Belichtung des Auges im Frequenzbereich zwischen 33 Hz und der Flimmerverschmelzungsfrequenz. *Vision Res.* 10: 1457–1469, 1970.

WERBLIN, F. Synaptic connections, receptive fields, and patterns of activity in the tiger salamander retina. *Invest. Ophthalmol. Vis. Sci.* 32: 459–483, 1991.

WEVER, E. G. *Theory of Hearing.* New York: Wiley, 1949.

XU, X. J., XU, J., HUANG, B., LIVSEY, C. T., AND KARWOSKI, C. J. Comparison of pharmacological agents (aspartate vs. aminophosphonobutyric plus kynurenic acids) to block synaptic transmission from retinal photoreceptors in frog. *Exp. Eye Res.* 52: 691–698, 1991.