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FLICKER FUSION CHARACTERISTICS OF ROD PHOTORECEPTORS IN THE TOAD

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Abstract—Critical fusion frequency (CFF) of toad rods was examined at various intensities using intracellular recordings. Data were compared to CFF measurements (as a function of intensity) obtained with the electroretinogram (ERG) and with a horizontal cell. In all instances the rod (and rod mediated) responses produced a curve which increased monotonically to a high frequency asymptote at about 6 Hz. The curves obtained with the ERG and the horizontal cell were double branched. The upper branch exhibited a Purkinje shift and thus must have been produced by cones. Even when using a red background to suppress cone sensitivity, there was no sign of a second rod mediated branch in the CFF curves from this retina.

INTRODUCTION

The critical fusion frequency (CFF) is the rate at which a light presented repetitively ceases to evoke flickering responses. In general, the more intense the stimulus the higher the CFF. When CFF is plotted against stimulus intensity it frequently is a curve with two separate smooth branches. This traditionally is an indication of the presence of both rods and cones. At low intensities responses are mediated by the rods; while rods are very sensitive, they are slow and consequently the CFF is low. As stimulus intensity is increased, a point is reached where responses begin to be mediated by cones and the CFF rises sharply (Hecht, 1937; Walls, 1942).

A kink in the CFF intensity curve need not imply cone mediated responses. Green and Siegel (1975) working on the eye of the common skate, an elasmobranch with an all rod retina, found double branched CFF curves when recording electroretinograms, S-potentials and massed receptor potentials. Two years later a psychophysical study in man demonstrated a similar double branched relationship for human rods (Conner and MacLeod, 1977). In both studies, spectral sensitivity determinations showed that the receptors producing the two branches contained the same rod pigment. Unanswered, however, was the question of whether the ability to follow rapid flicker is a property of all the rods in these retinas or represents the responses of a subset of less sensitive rods with unusually rapid kinetics.

One obvious way to determine what happens is to record from individual rods. For technical reasons it has not been possible to obtain stable intracellular recordings from skate rods. Consequently, we have recorded from *Bufo marinus* rods. The rods of this animal are large and readily accessible to intracellular recording. Because toad rods, like skate rods, initially saturate and then recover sensitivity during prolonged bright light exposure (Fain, 1976), we anticipated finding two branched flicker curves. Despite this expectation, the studies we report here indicate that the rhodopsin rods of the marine toad form a single class of photoreceptors that do not dramatically change their response characteristics.

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METHODS

The animals were placed in a brightly illuminated terrarium for 2–4 hr, then transferred to the dark for 30 min before pithed and having their eyes excised and hemisected under dim red light. The initial period of light adaptation helped to minimize the chances of retinal detachment. A portion of the vitreous was removed using absorbent paper wicks, the eye cup placed in a standard recording chamber through which moist 100% O_2 was gently blown and the eye cup dark adapted for an additional hour.

Uniform illumination of the eye cup was achieved through a conventional two channel photostimulator (Oakley and Green, 1976). The incident flux was measured in both beams at all wavelengths using a calibrated PIN-10 photodiode (United Detector Technology). To obtain the rate of photon absorption the measured flux at 500 nm was multiplied by 24 μm^2, the effective cross-sectional area of the rod obtained by taking rod cross-sectional area as 47 μm^2, mean absorption as 77% and quantum efficiency of bleaching as 0.67 (Fain, 1975). Square-wave flickering stimuli were produced using a rotating sectored disk. The retina was allowed to adapt for 5–7 min at each stimulus intensity. Following this, critical fusion frequency (CFF) was determined by increasing the frequency of stimulation until the response disappeared in the base-line noise of the recording (0.5 mV peak-to-peak for intracellular

recordings and 10 μV peak-to-peak for electroretinogram recordings: ERGs). Stimulus intensity was increased stepwise and CFF data recorded at each intensity. In some ERG experiments, a steady red background (650 nm, 50 nm half bandwidth or 625 nm; 10 nm half bandwidth interferance filters) superimposed upon the flickering stimuli was used to preferentially adapt cones. *B*-wave responses to dim, brief, flashed 500 and 600 nm stimuli were scotopically equated in the presence of a dim red background. The ratio of the steady red background intensity to the scotopically equated flickering test (500 and 600 nm) intensity was kept constant. That is, each time test intensity was increased background intensity was increased by the same factor.

Intracellular recordings were made with high resistance (150–300 MΩ) glass micropipettes (Omega Dot) filled with 2 M potassium acetate. Rhodopsin-containing rods were distinguished from other cells by retinal depth of recording, by the characteristic shapes of responses to brief (100 msec) flashes and by using scotopically equated 500 and 600 nm stimuli. Rhodopsin rods responded identically to scotopically equated stimuli at both low and high intensities while horizontal cells responded to bright stimuli with smaller responses to 500 than to 600 nm. The reverse was true for the one short wavelength ("green") rod we encountered (i.e. it responded better to 500 than 600 nm stimuli). Complete spectral sensitivity curves were obtained from several rhodopsin containing rods. In each case these curves peaked at 500 nm and confirmed the identification made on the basis of the two point (500–600 nm) spectral sensitivity test. Dark adapted rods responded equally to dim brief 500 nm and white light stimuli if 1.5 log neutral density was added to the white stimulus.

The vitreal electroretinogram (ERG) was monitored to assess the physiological condition of the retina during intracellular recording and to investigate the CFF function of the ERG *b*-wave. Recordings were made with blunt, low resistance (about 5 MΩ) Ringer-agar pipettes. The *b*-wave intensity response function was measured with maximum *b*-waves ranging from 310 to 790 μV.

RESULTS

Rod intracellular recordings

Intracellular recordings were made from 44 cells identified as rhodopsin rods. Critical fusion frequency measurements were made from a total of 7 rods which responded to brief bright stimuli with 15 mV or greater hyperpolarization and which were held long enough to make flicker measurements at two or more intensities (30 min or longer). Two different stimulus conditions were used. Data were obtained with white light flicker from 4 rods (Fig. 1: solid symbols) over 7 log units of intensity. Similar measurements were made with 500 nm stimuli from 3 other rods (Fig. 1: open symbols). Both stimulus conditions yielded the same result. Critical flicker frequency in rhodopsin-containing rods was a monotonically increasing function of light intensity. Maximum CFF ranged from 4.2 to 6.7 Hz for individual rods.

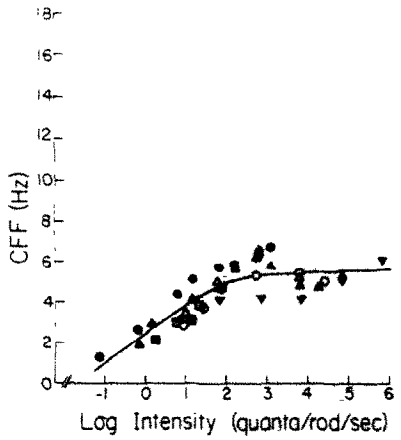

Fig. 1. Critical fusion frequency (CFF) vs stimulus intensity for rhodopsin rod photoreceptors. Each symbol represents the CFF response of a single rod to on-off flickering stimuli at 2 or more light intensities. Solid symbols indicate white light data and open symbols represent 500 nm flicker. CFF data obtained with white light stimuli are plotted against the effective 500 nm quantal absorbance of the rhodopsin rod photoreceptors (see text for details)

Horizontal cell recordings

Intracellular recordings were made from 7 identified horizontal cells and CFF data were obtained from one horizontal cell (Fig. 2). Over the same range of intensities used with the rods, critical flicker fusion (CFF) was a two branched function. An

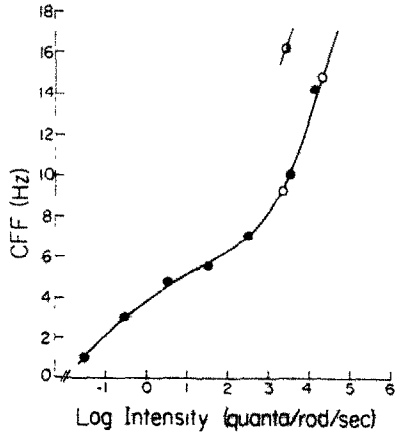

Fig. 2. Critical fusion frequency (CFF) vs stimulus intensity for a horizontal cell. Solid circles indicate white light flicker, open symbols represent CFF responses to 500 nm flicker and half filled symbol represents CFF response to 600 nm stimuli. The 500 and 600 nm stimuli were scotopically equated using the dark-adapted ERG *b*-wave. CFF data obtained with white light flicker are expressed in terms of the effective quantal absorbance in the rods

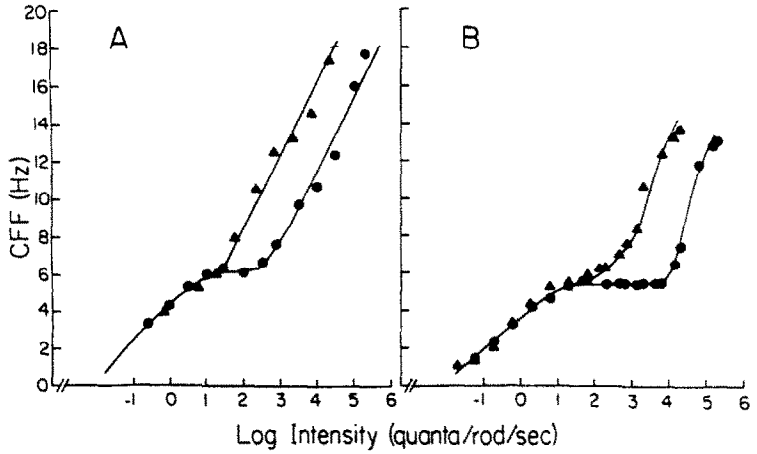

Fig. 3. Critical fusion frequency (CFF) vs stimulus intensity for the *b*-wave of the electroretinogram (ERG). (A) *Dark Adapted*: ERG frequency responses were measured as a function of light intensity using 500 nm (circles) and 600 nm (triangles) flickering stimuli against a dark background (DA). (B) *Modulated Flicker*: ERG frequency responses were measured as a function of light intensity using 500 nm (circles) and 600 nm (triangles) flickering stimuli in a fixed ratio to a red background (625 nm; 10 nm half band width). The ratio of test to background intensity in scotopic units was maintained at 2:1.

inflection in the horizontal cell CFF (Fig. 2: solid symbols) occurred at about 6.5 Hz (100 quanta/rod/sec) which corresponded to the plateau of CFFs obtained in individual rods. Increasing intensity above 1000 quanta/rod/sec yielded a dramatic increase in CFF. The maximum frequency obtained on the second branch was about 16 Hz. Horizontal cells in toad receive information from both rods and cones (Fain, 1976). Consequently, the spectral sensitivity of this second branch of the CFF function was examined using scotopically equated 500 and 600 nm flickering stimuli (Fig. 2; open circles vs half-solid circle). These data made the cone influence in the second branch apparent. Where the horizontal cell responded to fast flicker it was approx. 1.0 log unit more sensitive to 600 than to 500 nm stimuli. Thus, the intracellular data obtained from toad rods and horizontal cells were consistent with the classically held assumption that the rods respond to low frequency flickering lights and that cells which exhibit faster temporal properties receive cone input.

ERG recordings

The cells we have recorded from do not provide evidence for a fast flicker mechanism in rods. However, due to selection biases (see rod recordings) we might have missed a second class of rapidly responding rods. Consequently, we decided to conduct experiments using the electroretinogram (ERG) to establish whether a second, fast rod system might be uncovered.

CFF curves were obtained from ERGs recorded in 14 eyecups; 5 with no background (dark adapted), 5 with a fixed intensity long wavelength background (light adapted) and 4 with the background and test intensity in a fixed ratio (modulated flicker). Data from light adapted eyecups (LA) were intermediate between those obtained for dark adapted (DA) and eyecups presented with modulated flickering (MF) stimuli (see table). Figure 3A shows flicker data obtained with 500 and 600 nm on-off flickering stimuli from a dark adapted eyecup. These data should be compared with similar data obtained from modulated flicker (see Fig. 3B). In both conditions, the CFF-intensity functions had two branches and the responses to high intensity rapid flicker exhibited a Purkinje shift of about 1.0 log unit. Thus, the first branch reached a plateau at 6.0 Hz adapted and 5.5 Hz with modulated flicker. The only difference between the two conditions is that adding a red background in fixed ratio to the flickering stimulus, shifted the response of the cones to higher intensities. This extended the range of light intensities over which responses of the scotopic mechanism could be observed. With a 500 nm stimulus the data in Fig. 3B show that the scotopic branch of the flicker function was a monotonic function to 1×10^4 quanta/rod/sec, stimulation. For 500 nm stimuli the rod-cone break occurred at an intensity of 2.9 log quanta/rod/sec in dark adapted eyecups and at a log unit higher intensity (3.9 log quanta/rod/sec) in eyecups tested with modulated flicker (see table). In every case where a second CFF branch was present there was a Purkinje shift of from 1.0 to 1.5 log units (see table). However, in one experiment (Experiment No. 13; table) no second branch was observed in the CFF function and at all intensities the spectral sensitivity was that of rods. Thus, the scotopic branch of the ERG flicker curve agreed with the results obtained from individual rods. The electroretinogram provided evidence for a second branch but in every instance it represented the cone activity.

Table 1 Summary data for all ERG experiments

Conditions	Experiment No	Rod plateau (Hz)	Purkinje shift ($\log_{10}$)	Rod-cone break (500 nm) (log quanta rod sec)
DA	1*	6.5	—	2.7
	2	7.5	1.4	2.7
	3*	7.5	—	3.5
	4	6.0	1.4	2.7
	5	5.0	1.2	2.8
	Mean values	6.5	1.3	2.9
LA	6	6.0	1.2	3.5
	7	9.0	1.0	2.7
	8	4.3	1.0	2.9
	9	6.0	1.1	2.8
	10	5.0	1.0	3.1
	Mean values	6.1	1.1	3.0
MF	11	5.5	1.1	3.8 (3:2)
	12	5.5	1.0	4.0 (2:1)
	13†	4.2	—	—(1:1)
	14	5.0	1.5	3.8 (1:1)
	Mean values	5.1	1.2	3.9

DA = dark adapted; LA = light adapted, MF = modulated flicker. For the MF condition the ratio of test intensity to background intensity for rods is shown in parentheses at the right. 625 nm (Experiment Nos. 11 and 12) and 650 nm (Experiment Nos. 13 and 14) backgrounds were used.

*White light flicker—values included in rod-cone break column were shifted (1.5 log quanta) to correspond to rod quantal absorbance for 500 nm flicker.

†No second branch was present.

DISCUSSION

We have been unable to demonstrate duplex flicker responses in toad rods. While this is essentially a negative finding, it does tell us important things about rod flicker. First, we now know that rods do not exhibit double branched flicker-intensity relationships in all eyes. Moreover, the fact that Bufo rods do not exhibit this property tends to exclude a number of processes that were candidates for producing double branched flicker.

Light adaptation shortens the duration of the rod response to brief flashes in toad (Bastian and Fain, 1979; Baylor *et al.*, 1979), in skate (Dowling and Ripps, 1971) and in other cold blooded vertebrates (Baylor and Hodgkin, 1974; Hood and Grover, 1974; Detwiler, Hodgkin and McNaughton, 1980). The increased speed contributes to the higher CFF obtained with brighter stimuli (shown in Fig. 1), but it does not produce a kink in the flicker-intensity relationship (Toyoda and Coles, 1975).

The recent findings of faster responses at the basal end of the outer segment than at the distal tip of toad rods (Baylor *et al.*, 1979) might suggest that fast flicker responses are generated in the base and slow flicker responses in the apex of the outer segment. Our data from the toad indicates that such differences do not cause the rods to respond to fast flicker in the way they do in skate and man.

Green and Siegel reported that the abrupt increase in CFF was associated with saturation of the response amplitude. That is, it appeared only after the rods had been exposed to a steady light that had driven the response to its maximum limits. Such stimuli initially make skate rods unresponsive, but then they recover with time (Dowling and Ripps, 1970, 1971). After recovery, skate rods are capable of following high frequency flicker (Green and Siegel, 1975). This led the above authors to suggest that the two processes were related. That is, the same mechanism responsible for shifting the skate rod's response range might also enhance its temporal resolution. Toad rods have the ability to recover from saturating stimuli (Fain, 1976). Several hundred quanta per sec produce maximum amplitude rod responses so that at the highest intensities shown in Fig. 1, the stimulus onset initially saturated the rod response. Recovery from saturation is not, however, accompanied by a dramatic increase in the rod's ability to respond to high frequency flicker. Fast flicker responses in toad seem to be associated exclusively with cones.

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