Rod-cone interaction in flicker perimetry

KENNETH R. ALEXANDER AND GERALD A. FISHMAN

From the Department of Ophthalmology, University of Illinois Eye and Ear Infirmary, Chicago

SUMMARY We have assessed the influence of the rod system on cone flicker sensitivity during flicker perimetry. For temporal frequencies above 18 Hz extrafoveal cone-mediated flicker thresholds for a white test stimulus are as much as 1.5 log units lower when measured against a large background light that saturates the rods than when measured in darkness. Following a Ganzfeld bleach extrafoveal cone flicker thresholds are at their minimum once the cones have recovered their sensitivity, but then thresholds rise as the rods begin to recover from the bleach. Our results indicate that the flicker sensitivity of the extrafoveal cone system at high temporal frequencies is influenced by the rods surrounding the flickering test stimulus. The rods reduce flicker sensitivity maximally in the dark adapted state, and their suppressive influence is minimised only by strong rod bleaches or by large backgrounds that saturate the rod system.

Flicker perimetry has been used to assess the sensitivity of the human cone mechanism both in the normal visual system^{1–3} and in patients with visual abnormalities.^{4–6} Cone flicker sensitivity is usually measured by maintaining a constant luminance and decreasing the flicker frequency from a high rate until the flicker can just be detected (the CFF), or by maintaining a constant high frequency (usually at approximately 25 Hz) while the minimum luminance necessary to detect flicker is determined.

It has generally been assumed that the rod system does not influence cone flicker sensitivity at the high temporal frequencies used in flicker perimetry owing to an inability of the rod system to follow high rates of flicker. However, recent reports^{7,8} have indicated that the rod system can reduce cone flicker sensitivity at high frequencies if measurements are made in the dark adapted eye. These studies of rod-cone interaction in flicker sensitivity were performed in or near the fovea by means of chromatic stimuli. We have examined the generality of this rod-cone interaction in flicker perimetry by using a white flickering test stimulus presented at locations across the visual field. The threshold luminance for detecting flicker was measured both in the dark adapted eye and with rod sensitivity depressed by a background light or by a bleach. If the rod system reduces cone flicker sensitivity in the dark adapted eye, as suggested by the

previous studies,^{7,8} then light adapting or bleaching the rods should improve cone flicker sensitivity at all extrafoveal locations.

Materials and methods

SUBJECTS

Five naive observers ranging from 21 to 32 years of age participated in the experiments. All had normal (corrected) acuity and colour vision. During the experiments the subjects' pupils were dilated and accommodation was paralysed by the instillation of 10% phenylephrine and 1% cyclopentolate drops. Except where noted no optical corrections were used, since photopic flicker sensitivity at high frequencies is relatively unaffected by optical blur.⁹

APPARATUS

Testing was performed with a Tubinger perimeter. The circular test stimulus (TS) was either 104 min or 66 min in diameter and was either white (tungsten), red (Oculus glass filter, bandpass >620 nm), or blue-green (Oculus interference filter, $\lambda_{\text{max}} = 500 \text{ nm}$). The TS was presented against the perimeter diffusing surface at a number of retinal locations relative to a 30-min diameter dim red fixation target. For foveal testing the fixation dot was replaced by a diamond shaped pattern of 4 red dots 10 min in diameter and separated by 2°. Under certain of the experimental conditions described below the Tubinger fixation channel was used to present background fields rather

Correspondence to Dr Kenneth R. Alexander, University of Illinois Eye and Ear Infirmary, 1855 W Taylor Street, Chicago, IL 60612, USA.

than a fixation target, and fixation was controlled by an auxiliary dim red cross projected on to the appropriate location within the perimeter bowl. Under these conditions the subject fixated the laterally placed fixation target while maintaining a forward head position in the chin rest.

Temporal square wave modulation of the TS was provided by an episcotister rotating in a collimated portion of the optical path. The TS was presented for 3 seconds either in the dark, against the perimeter Ganzfeld of $0.5 \log \text{cd m}^{-2}$ or against a background provided by the fixation channel. The luminance of the TS was changed in $0.1 \log$ unit steps by a series of neutral density filters. For the bleaching experiments the subject's eye was exposed for 3 minutes to a Ganzfeld light of $3.6 \log \text{cd m}^{-2}$ provided by a Feldman Adaptometer (American Optical Co.). Stimulus luminances were calibrated by a Spectra Spotmeter. Flicker frequencies were checked by a photocell and oscilloscope. The photopic and scotopic values of the chromatic stimuli were calculated from the nominal colour temperature of the tungsten

source, the spectral transmission of the various filters (as determined by a Carey spectrophotometer), and the photopic or scotopic luminosity function. The calculated photopic values agreed with the relative threshold values obtained for these stimuli during the cone plateau portion of dark adaptation. The calculated scotopic values were equivalent to those obtained from absolute threshold measurements.

GENERAL PROCEDURE

Prior to an experimental session each subject was dark adapted for 45 minutes. During the session flicker thresholds were measured by determining the minimum luminance necessary to detect flicker in a temporally modulated light of a constant frequency (usually 25 Hz). For each trial the luminance was initially set below the flicker threshold to avoid flicker adaptation, and then the luminance was increased in $0.1 \log$ unit steps until the subject responded, by means of a buzzer, that flicker was detected (ascending method of limits). Threshold was defined as the median of 3 repetitions of this procedure.

Results

LIGHT ADAPTATION AND FLICKER SENSITIVITY

The threshold luminance for a 25 Hz, 104-min white flickering TS was measured at selected locations across the horizontal meridian of the visual field, first in the dark adapted eye, and then against the standard Tubinger Ganzfeld background of $0.5 \log \text{cd m}^{-2}$. The results for 3 observers are shown in Fig. 1. In the fovea the flicker threshold is relatively unaffected by the presence of the background field. However, in the retinal periphery flicker thresholds are as much as $1.5 \log$ units lower in the presence of the background light than in the dark.

To determine whether the improved flicker sensitivity of the light adapted eye is characteristic only at a frequency of 25 Hz we measured flicker thresholds at 20° in the temporal retina at frequencies from 2.5 to 40 Hz in steps of either 2.5 or 5 Hz. The results for one subject are shown in Fig. 2. At low temporal frequencies the background field elevates flicker thresholds considerably from the dark adapted level as expected from Weber's law. However, at temporal frequencies above 18 Hz flicker thresholds are consistently lower in the presence of the background than in the dark. Therefore, the improved flicker sensitivity in the presence of the background is not limited to 25 Hz but extends from 18 to at least 40 Hz.

To determine whether cones or rods mediate flicker thresholds under these conditions we measured flicker thresholds at 5° from the fixation point in the temporal retina for a 104-min TS that was

Fig. 1 Threshold luminances for flicker detection for 3 observers (N_1 , N_2 , N_3) using a 104-min diameter 25 Hz white test stimulus presented either in darkness (filled circles) or against a Ganzfeld background of $0.5 \log \text{cd m}^{-2}$ (open circles) at the retinal locations indicated.

Fig. 2 Threshold luminances for flicker detection for one observer (N_3) at the temporal frequencies indicated using a 104-min diameter white test stimulus presented at an eccentricity of 20° (temporal retina) either in darkness (filled circles) or against a Ganzfeld background of $0.5 \log \text{cd m}^{-2}$ (open circles).

either of a middle (BG) or a long (R) wavelength. Thresholds were measured at a number of temporal frequencies both in the dark adapted eye and against the Ganzfeld background. The results for 2 subjects are shown in Fig. 3. Thresholds are plotted in photopic units so that if cones alone are detecting the flicker then the thresholds for the 2 chromatic stimuli will be equal. If rods alone are detecting the flicker then the threshold for the R TS should be 2.5 log units higher than the threshold for the BG TS.

Fig. 3 Threshold luminances for flicker detection for 2 observers (N_1 , N_3) at the temporal frequencies indicated using a 104-min diameter 25 Hz test stimulus that was either of a long wavelength (squares) or middle wavelength (circles) presented either in darkness (filled symbols) or against a Ganzfeld background of $0.5 \log \text{cd m}^{-2}$ (open symbols) at an eccentricity of 5° (temporal retina).

As shown in Fig. 3, flicker thresholds for the 2 chromatic stimuli are virtually the same against the Ganzfeld background, demonstrating that light adapted flicker thresholds are mediated by the cone system. In the dark adapted eye flicker thresholds for the 2 chromatic stimuli are unequal at both low and high frequencies. At the lowest temporal frequencies the threshold difference corresponds to that of the rod system. At slightly higher frequencies the threshold difference becomes less than 2.5 log units, which implies that cones mediate flicker detection for the R TS while rods detect the flickering BG TS. At still higher frequencies (above 15 Hz) there remains a consistent difference between the thresholds for the 2 photopically matched stimuli in the dark adapted eye. Since it is unlikely that rods are detecting the flicker at these high frequencies (see Discussion), the difference in thresholds for the photopically matched test stimuli is likely to be due to the influence of rods on cone flicker sensitivity.

The Ganzfeld background light-adapts regions of retina that are far removed from the flickering TS as well as the retina on which the TS falls. To determine the actual retinal locus that must be light adapted to improve flicker sensitivity, flicker thresholds were measured at an eccentricity of 5° against steady white backgrounds of various diameters and of the same luminance as the Ganzfeld background. These backgrounds were provided by the Tubinger fixation channel and were presented in a random order. The TS was reduced in size to 66 min, and subjects were optically corrected for refractive error and target distance using trial lenses.

The effect of background diameter on flicker

Fig. 4 Threshold luminances for flicker detection for 3 observers (N_1 , N_4 , N_5) against steady white backgrounds of various diameters and a luminance of $0.5 \log \text{cd m}^{-2}$ using a 66-min diameter, 25 Hz white test stimulus, at an eccentricity of 5° (temporal retina). Solid line connects the means of the data points.

thresholds is shown for 3 subjects in Fig. 4. If flicker thresholds are measured against backgrounds that are smaller than or equal in size to the TS, flicker sensitivity is equivalent to that measured in the dark adapted eye. However, as the background is enlarged to light-adapt regions of retina outside the TS, flicker sensitivity improves. Therefore, it is the retina surrounding the TS that influences cone mediated flicker sensitivity.

The luminance of the background used to light-adapt the eye was $0.5 \log \text{cd m}^{-2}$ ($2.3 \log \text{scot td}$ on

Fig. 5 Threshold luminances for flicker detection for 2 observers (N_3 , N_5) against a steady white background 11° in diameter and at the luminances indicated at an eccentricity of 20° (temporal retina) using a 104-min diameter 25 Hz test stimulus. Solid line connects the means of the data points. Open circles represent detection thresholds for a 104-min diameter, 500 ms duration, 500 nm test stimulus against the same background. Solid line is fit by eye to the data points.

the assumption of a pupil diameter of 8 mm), which is near the level at which the rod system begins to saturate.¹⁰ To determine the effect of other background luminances on flicker sensitivity the 25 Hz white TS was presented in the centre of a background 11° in diameter at an eccentricity of 20° in the temporal retina. The background was systematically increased in luminance, and flicker thresholds were measured at each background luminance.

The results for 2 subjects are shown in Fig. 5 (triangles). As the background is increased in luminance there is little effect on flicker thresholds until the luminance is approximately $-2 \log \text{cd m}^{-2}$. The background luminance that has the greatest threshold lowering effect corresponds to the Ganzfeld luminance used in the previous experiments. At background luminances higher than this value flicker thresholds become elevated owing to the increasing light adaptation of the cone system.¹¹

Also shown in Fig. 5 is an increment threshold function against this same background for a 500 nm TS, 104 min in diameter, and flashed for 500 ms. All but the 2 highest data points are rod determined thresholds as indicated by an abbreviated spectral sensitivity measurement at each background luminance and by the presence of a rod-cone break in the dark adaptation curves for this TS against the various background luminances. From Fig. 5 it is apparent that the background begins to elevate rod increment thresholds when it is very dim, yet there is little effect on cone mediated flicker thresholds until

Fig. 6 Threshold luminances for flicker detection for 2 observers (N_1 , N_3) following a 3 min Ganzfeld bleach using a 104-min diameter 25 Hz white test stimulus presented either in darkness (filled circles) or against a Ganzfeld background of $0.5 \log \text{cd m}^{-2}$ (open circles) at an eccentricity of 20° (temporal retina).

Fig. 7 Triangles represent threshold luminances for flicker detection measured in darkness following the Ganzfeld bleach for 2 observers (N_1 , N_3), replotted from Fig. 6. Circles represent luminance thresholds for 2 observers (N_2 , N_3) under the same conditions as in Fig. 6 except that the test stimulus was flashed for 500 ms rather than flickered at 25 Hz.

the background luminance nears the level of rod saturation.

BLEACHING AND FLICKER SENSITIVITY

After a 3-minute Ganzfeld bleach, flicker thresholds for a 25 Hz 104 min white TS were measured at 20° in the temporal retina either in darkness or against the standard Ganzfeld background. The results for 2 subjects are presented in Fig. 6. For both subjects flicker thresholds initially following the bleach offset are the same whether measured in the dark or against the background. Thresholds against the background then remain constant over the next 20 minutes. In the dark, however, flicker thresholds begin to rise after 2–3 minutes and reach an asymptote at approximately 15 minutes.

A comparison of flicker dark adaptation with a more typical dark adaptation curve is shown in Fig. 7.

The circles represent luminance thresholds for the 104-min white TS flashed for 500 ms. The thresholds were measured by an ascending method of limits, and each data point represents the threshold value for one ascending series. The triangles are the flicker dark adaptation functions replotted from Fig. 6. It is apparent from Fig. 7 that flicker thresholds first begin to rise only during the cone plateau period of dark adaptation. Since the cones have fully recovered their sensitivity during this period, the elevation of the flicker thresholds is apparently due to the rapidly recovering rods.

There is an asymptote in the flicker thresholds at approximately 15 minutes following the bleach offset before the rods have fully recovered their dark adapted sensitivity. This implies that the rods do not have to be fully dark adapted to elevate cone flicker thresholds maximally. Furthermore, as shown in Fig.

7, the effect of the rods on cone flicker thresholds begins to lessen only when the bleach has reduced rod sensitivity by 2 log units or more. This is consistent with the finding presented earlier (Fig. 5) that rod sensitivity needs to be reduced by approximately 2 log units by a background light before cone flicker sensitivity improves.

Discussion

For temporal frequencies above 18 Hz cone flicker thresholds in the parafoveal and peripheral retina are lower in the presence of a background light than in the dark adapted state. This result is consistent with previous reports that the CFF is higher when the eye is light adapted.^{12,13} We have shown (1) that the reduction in flicker thresholds with light adaptation is minimal in the fovea but can be as large as 1.5 log units at greater eccentricities, (2) that the improved flicker sensitivity with light adaptation does not begin until rod thresholds have been elevated by at least 2 log units, (3) that the effect of the background light is maximal at background luminances that saturate the rod system, and (4) that the background light must be larger than the flickering test stimulus to improve flicker sensitivity. Furthermore, we have also found that, following a Ganzfeld bleach, flicker thresholds are at a minimum once the cones have regained their sensitivity. Then flicker thresholds become systematically raised as the rods recover from the bleach. These results are consistent with recent reports of rod-cone interactions in the detection of high frequencies of flicker.^{7,8} In those studies bleaching or light adapting the rods near the fovea improved cone flicker sensitivity. Our results suggest that rods have a significant effect on cone flicker sensitivity throughout the extrafoveal retina under conditions used in flicker perimetry. The influence of rods is minimised only by strong rod bleaches or by backgrounds that saturate the rod system.

There are several possible ways in which the rod system could interfere with flicker detection in the dark adapted eye. One possibility is that the rods that are stimulated by the high frequency flicker may generate a temporally modulated signal that is time shifted with respect to the cone signal and impairs temporal sensitivity through destructive interference, as has been reported at lower temporal frequencies.^{14,15} Two considerations suggest that this is not the case. First, we measured flicker thresholds in a subject with complete achromatopsia to determine whether the rod system is capable of detecting high frequencies of flicker under the conditions used in these experiments. Although this subject had a normal rod flicker response at low temporal frequencies, he was unable to detect flicker at a frequency

of 25 Hz anywhere in the visual field whether the eye was light adapted or dark adapted. This result is in agreement with previous reports of reduced flicker sensitivity in complete achromatopsia^{16,17} and is consistent with the finding that the rod electroretinogram (ERG) in the normal eye does not respond to temporal frequencies above 18 Hz.¹⁸ Second, it has recently been demonstrated that the reduced flicker sensitivity of the dark adapted eye occurs whether the light input to the rods is temporally modulated or steady.⁷ Therefore, even though the rod system of the normal eye can apparently respond to high frequencies of flicker under specialised conditions,¹⁹ it seems unlikely that it is a temporally modulated signal from rods that reduces the flicker sensitivity of the cone system under the conditions studied here.

A second possibility is that a steady rod signal generated by the flickering TS reduces the amplitude of the cone signal and thereby reduces flicker sensitivity. However, if such were the case, a middle wavelength TS should elevate dark adapted flicker thresholds more than a long wavelength TS, since the middle wavelength TS produces a larger rod signal. Yet, as shown in Fig. 3, dark adapted flicker thresholds are higher for the long wavelength TS which is in agreement with a previous report.²⁰ Second, it is difficult to remove such a steady rod signal, but it is easy to add to it by superimposing the flickering TS on a steady background of the same diameter as the TS. As shown in Fig. 4, flicker thresholds against a background of the same size as the TS are equivalent to thresholds in the dark adapted eye. It is only when the background is larger than the TS that flicker sensitivity is affected. Therefore it appears unlikely that a steady signal from the rods underlying the TS is responsible for the reduction of flicker sensitivity in the dark adapted eye at the temporal frequencies used in these experiments.

A third possible explanation is that the rods surrounding the TS in the dark adapted eye laterally inhibit the cones, reducing their flicker sensitivity. Such an explanation was originally proposed by Hood²¹ to account for an analogous finding in the photopic flicker ERG of the frog retina, and has recently been suggested as an explanation for human psychophysical results as well.⁷ This lateral inhibition is different from that typically encountered in receptive field measurements²² but is consistent with the finding that the maximum release of neurotransmitter by the photoreceptors occurs in the dark adapted state, as discussed by Hood²¹ and Goldberg *et al.*⁷

In conclusion, when cone flicker thresholds are measured in the extrafoveal visual field of the dark adapted eye, the rods surrounding the test stimulus appear to reduce appreciably the flicker sensitivity of the cone system. The inhibitory effect of the rods

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begins to lessen only when rod sensitivity is depressed by at least 2 log units either by a sufficiently intense large background field or by a bleach. The influence of the rods is least and thus flicker sensitivity is maximal during the first few minutes following a bleach or against a large background field that saturates the rod system. In studies of patients with retinal dysfunction to be reported in a subsequent article we have found that this interaction between rods and cones in flicker perimetry can be abnormal, suggesting that a comparison of dark adapted and light adapted flicker thresholds may be a useful test of visual function for the extrafoveal retina.

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