Response of the retina at low temporal frequencies

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We investigated the low-frequency temporal response of the retina by measuring the corneal electroretinogram elicited by flickering lights. A sum of two temporal sine-wave modulations was used to generate difference frequencies between a 36-Hz standard stimulus and a series of low-frequency stimuli. The response of the retina at the difference frequency did not change as the low-frequency component of the stimulus was varied from 0.5 to 4 Hz. We also replicated an earlier study, stimulating the retina with a sum of two sine waves that were varied in average frequency but keeping the difference frequency constant. These data showed no change in the amplitude of the difference frequency as the average stimulus frequency was varied from 8 to almost 40 Hz. Taken together, the two sets of data support the notion that the *in vivo* early retinal response is low pass and extends without attenuation to frequencies greater than 30 Hz, in contrast to the sensitivity of the visual system measured by psychophysical techniques.
Key words: electroretinogram, temporal response, vision, visual adaptation. © 1996 Optical Society of America

1. INTRODUCTION

The temporal response of the visual system is bandpass, with a peak sensitivity at 10–20 Hz and decreased sensitivity at both higher and lower temporal frequencies.[1–5] At high frequencies the decrease in sensitivity with increasing temporal frequency is attributable to limitations in the rate at which the nervous system can respond. As a result, the rate is thought to decrease with each stage of processing, as would be expected for a system of cascaded processes.[6] At low temporal frequencies the reduced sensitivity with decreasing frequency is generally attributed to both the process of light adaptation and the interaction of spatial and temporal processing. That is, the visual system filters out both slow changes in retinal illuminance and low-frequency noise, presumably to optimize its ability to detect rapid changes and spatial variations in luminance.

Previously we showed that there was an early stage of visual processing that was low pass,[7] i.e., that showed minimal attenuation at low temporal frequencies. We made these measurements by recording the corneal electroretinogram (ERG) elicited by stimuli that were composed of a sum of two temporal sine waves. In this method the frequency difference between the two sine waves is kept constant while the average stimulus frequency is varied. Because a response at the difference frequency is generated only by nonlinear processing, the amplitude of the difference signal depends on the ability of the visual system to transmit the two stimulus frequencies to the stage of the visual system that is nonlinear. Inasmuch as the difference between the two stimulus frequencies is held constant in frequency, the rest of the visual system cannot discriminate between the 8-Hz responses generated by stimulation with the sum of a 12- and a 20-Hz sine wave and the 8-Hz responses generated with the sum of a 56- and a 64-Hz sine wave. Although there are a number of assumptions involved in

this technique (see Section 4), it is a powerful tool for investigating visual information processing.[7–12]

In our previous study we stimulated the eye with the sum of two temporal sine waves that differed in frequency by 8 Hz. We found that the amplitude of the 8-Hz beat was constant as the average stimulus frequency was varied from 12 Hz to almost 40 Hz. This result implies that the early retinal response behaved like a low-pass temporal filter with a corner frequency in the vicinity of 40 Hz. We concluded that the nonlinearity that generates the nonlinear responses to flickering stimuli is in the distal retina. Furthermore, prior to the site of the nonlinearity, the retinal response was both fast and temporally low pass. We have also shown that the site of the nonlinearity recorded with the flicker ERG is located prior to the site of convergence of information from spectrally different classes of cones.[13] This site is generally assumed to be at the cone triad synapses, where information from photoreceptors, horizontal cells, and bipolar cells interact. Taken together, these results can be used to argue that the initial temporal response of the photoreceptors is both rapid, extending to high frequencies, and temporally low pass, showing little sign of decreased sensitivity at low temporal frequencies.

Schnapf and co-workers have found evidence that the response of isolated cone outer segments is temporally bandpass.[14] Because they recorded the current flow at the outer segments, one can raise the question whether the response reported by Burns *et al.*[7] is truly low pass, as their lowest stimulus frequency was at 12 Hz. In the current paper we reexamine this question, using a modification of the sum of two sine waves technique used by Burns *et al.*[7] There are three primary factors that control the frequency range that can be investigated with the flicker ERG. First, at least one of the stimulus frequencies has to be sufficiently high to minimize response components contributed by Müller cells.[15,16] If the nonneural elements are too slow to respond to one of the stimulus

frequencies, then they will not contribute to the generation of nonlinear sums and differences between the two stimulus frequencies. Second, the difference frequency has to provide an adequate signal-to-noise (S/N) ratio. Because the noise in the ERG rises rapidly at low temporal frequencies,[7] the use of two closely spaced stimulus frequencies would result in a low-frequency difference response and an inadequate S/N ratio. In addition, if the stimulus frequencies are too far apart, then any temporal filtering, such as an early bandpass filter, may differentially attenuate the response at the two frequencies, resulting in a poor estimate of the initial linear filter. Previously the lowest-frequency pair that we used was the sum of a 12- and a 20-Hz sinusoid.

In the present study we investigate the temporal sensitivity of the early retina, using two techniques to measure the frequency response of the initial stages of the retina. Each technique is appropriate for a different range of stimulus frequencies. Both use a sum of two sine waves as the stimulus. The first approach is to keep the difference frequency at a constant temporal frequency. By varying the average stimulus frequency, we can measure the relative attenuation of the retina prior to the generation of the difference frequency by a nonlinearity. This approach has been widely used for studying both temporal[7,17,18] and spatial[8,9,19,20] visual processing. In the current study we use this technique over a broader frequency range than was used previously,[7] but we are still limited to fairly high temporal frequencies.

The second approach is designed to investigate the response at low temporal frequencies. We measure the response at the difference frequency generated by the nonlinear interaction between a 36-Hz standard stimulus and a variable low-frequency stimulus. If the frequency of the difference response is limited to a narrow range of frequencies (a few hertz), then the differential effect of variations in the retinal response with temporal frequency should be small, and the amplitude of the difference frequency should reflect the relative transmission of the variable frequency stimulus up to the nonlinearity that generates the difference frequency. Figure 1 depicts this approach. The solid curve is the amplitude response of the retina to sine-wave stimuli varying from 5 to 100 Hz.[7] The response amplitude first decreases to a minimum at $10-15$ Hz, presumably because of decreased contributions from rods and from Müller cells, which are limited in the rate at which they can respond. The response amplitude then increases with increasing frequency, rising to a broad peak at $30-40$ Hz.[7,21,22] Finally, the response amplitude decreases steadily at frequencies above 40 Hz. If we add a 0.5-Hz stimulus (F1) to the 36-Hz standard (Fs), then a difference frequency is recorded at 35.5 Hz (Fs − F1), and the amplitude of the difference-frequency response is related to the strength of interaction at the site of the nonlinearity. If the low-frequency stimulus is 2 Hz, then the difference frequency will be recorded at 34 Hz. As long as the retinal response following the nonlinearity is similar between 34 and 35.5 Hz, the relative strength of the nonlinear interaction between a 0.5- and a 2-Hz stimulus can be compared. Even though the 0.5-Hz stimulus may be eliciting responses from both inner retinal and nonneural response elements, the difference frequency must arise from elements that are capable of responding

to the 36-Hz stimulus component as well as to the 0.5-Hz stimulus component. Thus we would expect that, if adaptation occurred prior to the site of the nonlinearity, then we should measure a bandpass temporal-sensitivity function. If the adaptation follows the nonlinearity, or if the adaptation process itself is nonlinear, then we should measure a low-pass sensitivity function.

2. METHODS

A. Subjects
Two male subjects, both with normal retinas and normal color vision, were tested. All the protocols were approved by the Institutional Review Board of the Schepens Eye Research Institute.

B. Apparatus and Stimuli
The apparatus was the same as that used in Chang et al.[13] The stimulus was a 3500-troland 543-nm light that could be linearly modulated over a range of approximately 1000:1. The stimulus was the sum of two sine waves, generated by a programmable function generator, which in the current experiment was programmed to generate 2048 samples at a rate of 1024 Hz. Thus the entire stimulus was repeated every 2 s, giving us a minimum stimulus frequency of 0.5 Hz and a maximum stimulus frequency of 512 Hz. The time-average luminance and chromaticity were constant across all the stimulus conditions; thus each stimulus component comprised a sinusoidal modulation about the mean retinal illuminance.

The 40-deg stimulus field was surrounded by a ganzfeld to suppress the responses of the peripheral retina. Stimuli were the sum of two temporal sine waves. The modulation of both stimulus frequencies was 0.5, except where otherwise noted. We used two sets of stimuli. In the first set the stimulus was the sum of two temporal sine waves. The frequencies were always separated by 8 Hz, and the lower frequency was varied from 1 to 56 Hz.
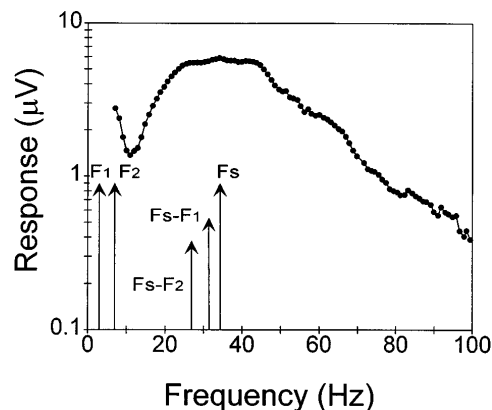

Fig. 1. Logic of the variable stimulus experiment. The retina is stimulated with the sum of a 36-Hz sine-wave stimulus (Fs) and a 0.5-Hz sine wave, and a response is generated at the difference frequency (Fs − F1), as well as at other sum and difference frequencies. If the low-frequency stimulus is changed to F2, then the difference-frequency response changes frequency (Fs − F2). As long as the difference frequencies are nearly the same, we can treat the response of later retinal processing as identical. Thus changes in the response amplitude with changes in the variable frequency must arise from differences in the transmission of the low-frequency response to the nonlinearity.

These conditions are essentially replications of the conditions used in experiment II of Burns et al.,[7] with the exception that a larger frequency range was used in the present experiment. In the second technique one of the sinusoidal components was always at 36 Hz, whereas the other was 0.5, 1.0, 2.0, 4.0, or 8.0 Hz. Both sets of data were recorded in a single session. Each subject repeated each main condition at least twice. We also included several control conditions in which we varied modulation, frequency, or both. Results from some of these control conditions are reported below.

C. Recording Conditions

Retinal responses were recorded by means of a Burian–Allen contact lens. The signal from the eye was amplified by two cascaded preamplifiers (Grass Instruments P511) and was digitized at 12 bits (Data Translation). The stimulus generators and the analog-to-digital converters were all run by a master clock to avoid mismatches between stimulus and recording timing. For each condition 4096 samples were acquired at a sampling rate of 256 Hz for a total recording time of 16 s/condition. The entire data sample was analyzed without averaging. Each main stimulus condition was repeated at least twice, and the average amplitude was computed for each subject.

D. Analysis

The recorded ERG was processed with a discrete Fourier transform. The amplitude, the phase, and the S/N ratio were computed. The S/N ratio was estimated as the ratio of the magnitude of the response at the frequency of interest (the signal plus noise) to the average magnitude summed over adjacent, lower-frequency, bins (the noise).[7] Only results whose ratio of signal-plus-noise to signal is greater than 3 are reported.

3. RESULTS

The response at the difference frequency, as well as at the standard frequency, was significantly greater than the noise amplitude at adjacent frequencies. Figure 2 shows examples from a single session of the magnitude of the discrete Fourier transform of the flicker ERG when the low-frequency stimulus was 1.0, 2.0, 4.0, or 8.0 Hz (bottom to top, respectively). The curves have been vertically displaced for clarity. Note that there is little change in the amplitude of the difference frequency until the difference frequency is 28 Hz (an 8-Hz difference from the standard). From Fig. 1 it is clear that below 30 Hz the amplitude response of the total retinal response to sine-wave flicker changes rapidly with flicker frequency; thus, with variable stimuli above 6 Hz (and with difference frequencies below 30 Hz), the assumption that the retina treats all the difference frequencies equivalently is violated.

The average results obtained with both methods are shown for each of the subjects represented in Fig. 3. Results obtained with the first method (with the difference frequency kept constant) are similar to those reported by Burns et al.[7] and are shown in Fig. 3(a). The amplitude of the 8-Hz beat is plotted as a function of the average of the two stimulus frequencies. Thus the 8-Hz response to a sum of a 16- and a 24-Hz stimulus would

be plotted at 20 Hz. For both subjects the amplitude of the 8-Hz response component is unchanged as the average stimulus frequency is varied from 9 to 36 Hz. Above 40 Hz the amplitude of the 8-Hz difference component drops rapidly. There is also a decline in the amplitude of the 8-Hz response at low stimulus frequencies. This decline in amplitude occurs when the stimulus frequencies are 1 and 9 Hz, a condition that is expected to generate responses from both the inner retinal neurons and the Müller cells.[23,24] We have also shown that in this frequency range there are strong nonlinear cone opponent responses in the ERG responses.[13]

Results obtained with the second method are plotted in Fig. 3(b). As the variable stimulus frequency was changed from 0.5 to 4 Hz the amplitude of the difference frequency did not change. To ensure that this insensitivity to the stimulus conditions was not due to response saturation, we also recorded responses when the modulation of both stimulus frequencies was reduced to 25%. In this case there was an approximately fivefold reduction in the amplitude of the beat,[25] indicating that the absence of a change in response amplitude at the difference frequency is not a consequence of response saturation.

4. DISCUSSION

The results of the current study support the idea that, prior to any nonlinear retinal processing, the response of the retina is temporally low pass and essentially flat from 0.5 to 40 Hz. This result has been replicated and extended from a previous study and in fact is evident in the data of Chang et al.[13] which were collected under different stimulus conditions. To reach this conclusion we have implicitly adopted a sandwich model of visual
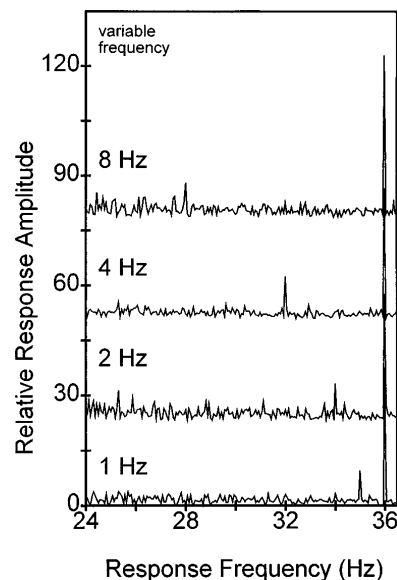

Fig. 2. Magnitude of the discrete Fourier transform from 24 to 36 Hz when the variable frequency stimulus was either 1, 2, 4, or 8 Hz (bottom to top, respectively). There are reliable response components both at 36 Hz (Fs) and at the difference frequency. Whereas the response amplitude at the difference frequency is relatively unchanged as the variable frequency changed from 1 to 4 Hz, it decreased for the 8-Hz condition (when Fs − F2 was 28 Hz). The responses have been displaced vertically for clarity of presentation.
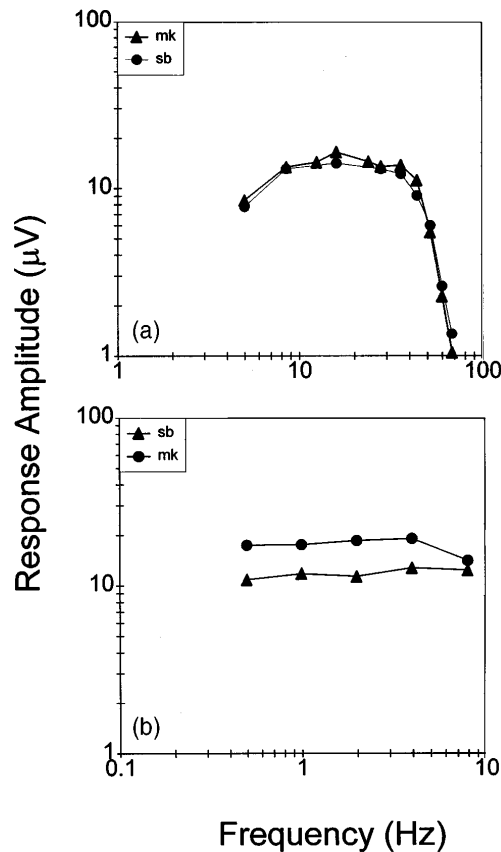
Fig. 3.   Results for both conditions for each subject.   (a) Plot of results of the standard technique for the sum of two sine waves for each observer.   In this technique the difference frequency was always at 8 Hz, and the amplitude of the response at 8 Hz is plotted as a function of the average stimulus frequency.   (b) Plot of the amplitude of the ERG response at the difference frequency when the eye was stimulated with the sum of a low-frequency stimulus and a 36-Hz stimulus, both at modulations of 0.5.   The amplitude at the difference frequency is plotted as a function of the frequency of the low-frequency stimulus.

processing.[12]   A sandwich model includes three sequential stages: an initial linear stage, a nonlinear stage, and a second linear stage.   When applicable, a sandwich model is a powerful tool for analyzing the behavior of a nonlinear system because it allows the behavior of the system to be partitioned into sequential stages of processing. The application of the simplest form of a sandwich model involves two assumptions: first, that there are not multiple sites contributing to the generation of the nonlinear response components, each with its own interposed temporal response function; and, second, that the nature of the nonlinearity does not change with stimulus conditions (i.e., a static nonlinearity).   However, it is important to note that a sandwich model can be generalized to the description of more-complex systems.[26]   For the flicker ERG the conditions of the first assumption are likely to be met because the response arises mainly from the photoreceptors and the bipolar cells.   However, we have found that the retinal response does not fully meet the conditions of the second assumption in that the nonlinearity is not static.   For sine-wave stimuli there is a power-law relation between stimulus modulation and retinal response amplitude, and the exponent of the power law depends on both the frequency of the stimulus and the stimu-

lus history of the retina.[27]   However, it is unlikely that this deviation from static behavior could alter our conclusions.   If the retinal response properties were changing with changes in the stimulus, i.e., the retinal response departed significantly from static behavior, then the retinal response would be expected to change with stimulus conditions.   This effect did not occur; the response amplitudes stayed the same.   This result is consistent with our previous experiments,[27] inasmuch as we found that the departures from a static nonlinearity are decreased at high modulations and with temporally complex stimuli, the conditions used in the current experiment.

We have chosen conditions to minimize the effect of the first assumption on our data.   Over most of the stimulus conditions at least one of the stimuli is above 20 Hz. For a difference response to be generated by a cell, the cell must respond to both frequencies.   It has been shown that the response to rapid flicker is maximal in the region of the photoreceptors and the bipolar cells,[23,28,29] and it is unlikely that glial cells can follow flicker at these frequencies.[15,24]   Thus the responses that we are measuring most likely arise from both the photoreceptors and the bipolar cells.[30,31]   In addition, Palm[26] has shown that a cascade of static nonlinear elements with interposed linear filters can be formally treated with a linear–nonlinear–linear sandwich model.   We conclude that our data support the hypothesis that we are measuring the properties of early retinal signal processing.

A.   Locus of Nonlinear Response Generation

The measured low-pass characteristics are an indication of what information is available at the site of the nonlinearity that generates the nonlinear response components of the flicker ERG.   Because the low-pass response is deduced from the total retinal response properties, we do not know at what stage of retinal processing it occurs.   We can measure only the response of the retina as a whole and conclude that this stage is early in the retina.   For the reasons cited above, we believe that the flicker ERG arises primarily from the photoreceptors and the bipolar cells.   In addition, there is physiological[14] and psychophysical[32] evidence that low-frequency attenuation occurs in the distal retina and probably is present in the response of the photoreceptors themselves.[14]   Thus the most logical conclusion is that the nonlinearity is located within the cones.   This conclusion is also consistent with the results of Chang, et al.[13] who showed that the nonlinearity occurred prior to the site of convergence of signals from different spectral classes of cones.   The most likely interpretation of the current results, taken together with the available literature, is that the temporal characteristics measured in this experiment reflect the kinetics of the initial stages of cone transduction.

B.   Overall Temporal Response of the Flicker ERG

The current data also address the relative attenuation of different frequencies after the first linear stage of the retinal response.   Assuming that there is little attenuation of a signal in the initial stages of visual processing between 0.5 and 40 Hz, then properties of the later stages can be deduced by examination of the fundamental response amplitude to a sine-wave stimulus.   Figure 4 compares the response amplitude at the frequency
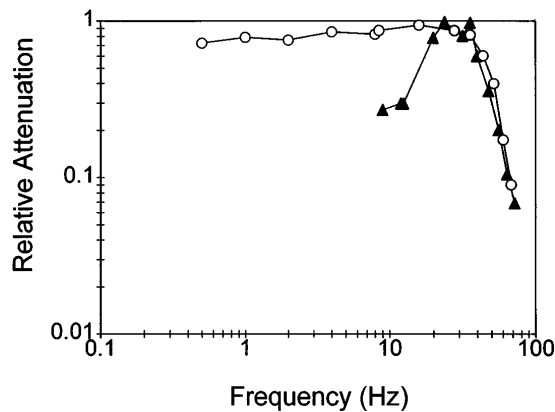
Fig. 4. Comparison of the frequency response deduced for the initial linear filter (open symbols) and the total retinal response (filled symbols). Results have been averaged across subjects and have been normalized to a value of 1.0 at their estimated peaks. Most results from conditions that are believed to violate the assumptions of the methods (see text) have been omitted from the average, although the results from both experiments at 8 Hz are included for completeness.

of stimulation (the first harmonic responses) with the response amplitude measured at the difference frequency, both having been measured within the same experimental sessions. Both curves are the average of two runs for the two observers, and their heights have been normalized. There is a large attenuation of the fundamental response component at low temporal frequencies, consistent with temporally dependent light adaptation. At high frequencies the two curves descend roughly in parallel. We have shown that at these frequencies there is a roughly linear relation between stimulus modulation and response amplitude.[27] Thus, at high temporal frequencies, most of the attenuation in response amplitude is occurring within the initial stages of the visual system response. Note that, if the psychophysical threshold were plotted on this same curve, it would decrease with frequency at a much higher rate.[13]

In conclusion, we have measured the temporal response of the early stages of retinal processing. At this stage the retina follows both slow and rapid changes, responding from 0.5 Hz to almost 40 Hz without decrement, and the response amplitude at 65 Hz has dropped to only one tenth of its peak sensitivity.

ACKNOWLEDGMENTS

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