The retinal response to sinusoidal variations in light intensity at very low frequency

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The frog retinal response to light is obtained in the frequency domain. The response is obtained by means of a capillary-type electrode at the cornea. Sinusoidal stimuli are used. The very low frequency range (0.01 to 1 Hz.) is examined. With decreasing frequency, retinal gain increases and the response is more nearly sinusoidal. The increase in sensitivity is thought to result from rod enhancement. The linear-nonlinear-linear transition occurring over the entire bandwidth of the frog retinal system is explained in terms of a two-component model.

Key words: retinal response, sinusoidal variations, low-frequency light intensity, steady-state ERG

The visual system has been described as having low-pass filter characteristics. It has poor low frequency response, good intermediate response, and a rapidly falling high frequency roll-off as indicated by amplitude gain versus frequency characteristic.[1] This is true of the spatial and temporal transfer functions in psychophysics[2, 3] and electrophysiologic studies of both the analogue and digital retinal outputs.[4-6] The low frequency cutoff has been attributed to lateral inhibition in both spatial and temporal domains.[3, 7] The high frequency spatial response is limited by the optical system,[8] and the temporal response for action potential–generating mechanisms is probably limited by the mechanics of impulse generation.[1]

Most studies of visual systems have investigated the frequency response from about 0.2 to 0.5 Hz. up to cutoff. Cutoff is defined as the frequency at which the response is approximately 70 per cent of maximum. In the Limulus, Ratliff and associates[9] and Knight and associates[10] examined the light stimulus to spike rate frequency response, finding that the gain falls off quickly below 1 Hz. and the system shows phase advance below this frequency. At about 5 Hz. there is a phase lag of 180 degrees. In cat ganglion cells the gain-frequency characteristic rises to maximum slowly, followed by a rapid decline.[4, 11] Psychophysical studies[12, 13] show similar characteristics for the modulation sensitivity-frequency characteristic.[2] Similar type characteristics have been found in the gain-frequency characteristics for on-cells, on-center units, and off-center units.[14]

In the electroretinogram (ERG), Pop-

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pele and Maffei[6] and Maffei and Poppele[5] have demonstrated the gain-frequency and phase-frequency characteristics for both the cat ERG and cat late receptor potential (LRP). The ERG shows flat intermediate and fast high frequency roll-off characteristics with a high frequency cutoff at 8 Hz. The system shows a 180 degree lag at about 5 Hz. The intraretinal ERG is 180 degrees advanced relative to the corneal ERG. It consequently shows phase advance below 5 Hz. The LRP has low-pass characteristics with an 18 db. per octave roll-off and a cutoff frequency of 1.5 Hz. The system shows phase advance up to 4 Hz. at a phase lag of 180 at about 5 Hz. These ERG systems have not been investigated at frequencies much below 1 Hz.

Models of the frog's ERG have been proposed[15, 16] that incorporate system nonlinearities to account for the transition from low frequency nonlinear responses to higher frequency linear response. In one case[17] the model consists of cascaded linear and nonlinear elements, while in the other case[16] two delays are incorporated into the model with a fixed interdelay time. Poppele and Maffei[5] found the cat ERG linear for low modulations, while Rodieck and Ford[18] found the cat ERG nonlinear even for incremental stimuli. In the frog, it was demonstrated that a reduction in the modulation depth resulted in little change of the nonlinear component in the ERG.[19]

The current study is a preliminary investigation of the lower frequency characteristics (0.01 to 1 Hz.) of the frog's retinal response to light. It treats the steady-state frequency response of the retina to sinusoidal light stimuli. This response we define as the steady-state ERG.

For the purpose of definition and clarity, the frequency range 0.01 to 0.5 Hz. will be termed the very low frequency range. From 0.5 to 3 Hz. will be termed the intermediate frequency range. Frequencies above 3 Hz. will fall in the high frequency range.

The sinusoidal light stimulus just fills the dilated pupil and the response is monitored at the cornea. The very low frequency range appears to the author to have been ignored because of the poor low frequency characteristics attributed to visual systems in general and the problem of direct current drift, which becomes an increasing nuisance with decreasing frequency. A further point to be considered is that very slow changes in light intensity might cause changes in the state of adaptation and therefore produce a change in the electrooculogram (EOG). However it is thought that such an influence is minimal and affects our data very little.

Methods

Frogs were curarized using 0.07 c.c. of 3 mg. per milliliter tubocurarine injected into the sacral area of the spinal cord. The frog was mounted on a special frame. The nictitating membrane of one eye was carefully removed and its pupil dilated with Neo-Synephrine. A wire under the eye caused it to bulge. It was then placed in a light-tight cage. Capillary-type silver-silver chloride electrodes were used to record responses from the cornea when the retina was stimulated with light. The indifferent electrode was placed behind the eye. Sinusoidal variations in the light level were obtained by use of a glow modulator tube (Sylvania R1131C) appropriately biased to provide a steady light level and then symmetrically modulated by a function generator (Hewlett-Packard 3300A), with a low frequency limit of 0.01 Hz.

The output of the glow modulator was monitored by a photocell. The amplitude control on the function generator enabled the peak-to-peak amplitude of the light stimulus to be adjusted. The maximum peak-to-peak amplitude was exactly twice the steady direct current light level. This corresponds to 100 per cent modulation or a modulation depth of unity. The glow modulator output was focussed so that the beam fell within the pupillary aperture approximately filling it. The recording electrode was placed on the pupillary border.

Recordings were not made until a time judged sufficient for dark adaptation to have elapsed. This usually was about one-half hour. A check of the level of dark adaptation was obtained, using the response to a step change in light level. When the response amplitudes taken at five-minute intervals were found to be equal, the retina was as-

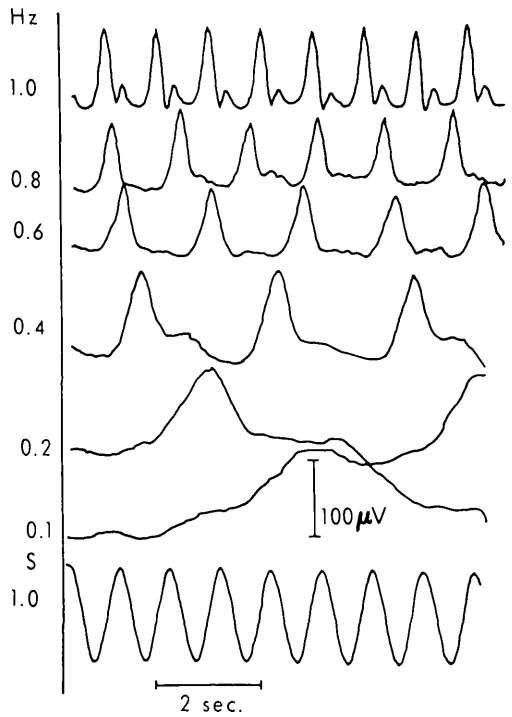

Fig. 1. Frequency response in the range 1 to 0.1 Hz. The stimulus, S, is shown at 1 Hz. Irrespective of stimulus frequency, the response starts in each case when the light intensity reaches maximum. The modulation depth is set just below 100 per cent. Note that with decreasing frequency the response for sinusoidal inputs becomes more sinusoidal. These recordings are single recordings measured at the corneal surface.

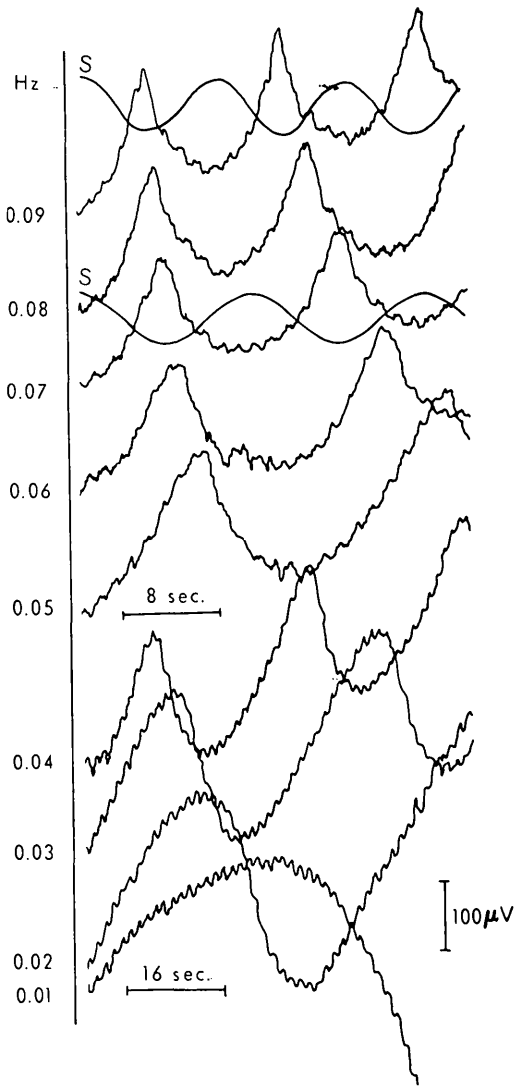

Fig. 2. Frequency response in the range 0.09 to 0.01 Hz. The stimulus, S, is shown at 0.09 Hz. and 0.07 Hz. The modulation depth is set just below 100 per cent. Note that the response for sinusoidal inputs is essentially sinusoidal. These recordings are single recordings measured at the corneal surface.

sumed to be dark adapted. Problems of drift frequently occurred, and if the response did not stabilize a new frog was prepared. At the very low frequencies, the system output must be free from drift to obtain stable recordings. By means of direct current-coupled amplification, responses were placed in the memory of a data-averaging computer (Nuclear Chicago 7100). The computer was triggered at the instant the light intensity reached its maximum value. Consequently, all response frequencies shown start as the sinusoidal light stimulus reaches maximum. For this reason only one stimulus waveform is provided in most diagrams. In general, single responses were obtained. However, use of the data-averaging computer enabled the response to be examined closely before recording. The contents of the memory were then read out on an XY recorder (Hewlett-Packard 2D-2).

Results

The experimental findings are demonstrated in the waveforms of Figs. 1 to 5. All recordings have been taken from the cornea, under conditions of dark adaptation at a modulation depth a little below 100 per cent. In Fig. 1 the corneal response in the frequency range 1 Hz. down to 0.1 Hz. is shown. In the first set of data (Fig. 1), the response at a frequency of 1 Hz. exhibits a large harmonic content. Its amplitude is about 100 μv.

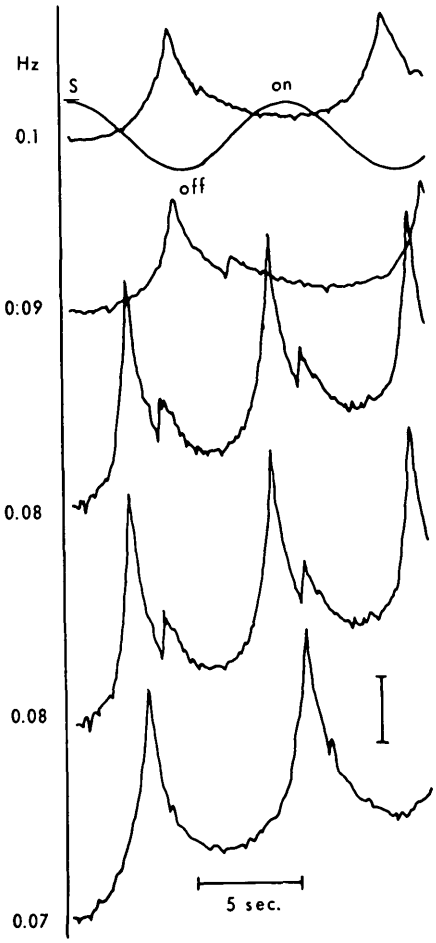

Fig. 3. Low frequency wave forms sometimes reveal the presence of a second harmonic. Here it is most prominent at 0.08 Hz. All records have been recorded at the same vertical sensitivity. The first two (0.1 and 0.09 Hz.) are single records, while the others are the result of two summations. S represents the stimulus. All recordings are taken from the corneal surface. The vertical calibration represents 100 μv for the first two waveforms and 200 μv for the rest.

With decreasing frequency the amplitude increases and the harmonic content decreases, until by about 0.1 Hz. the system response is approximately sinusoidal for sinusoidal inputs. In Fig. 2 the corneal frequency response is shown in the range 0.09 Hz. down to 0.01 Hz. when the period is 100 seconds. The amplitude is about 150 μv for the upper frequency and about 300 μv for the very lowest frequency. Again the output is approximately sinusoidal. Fig. 3 indicates corneal frequency responses in

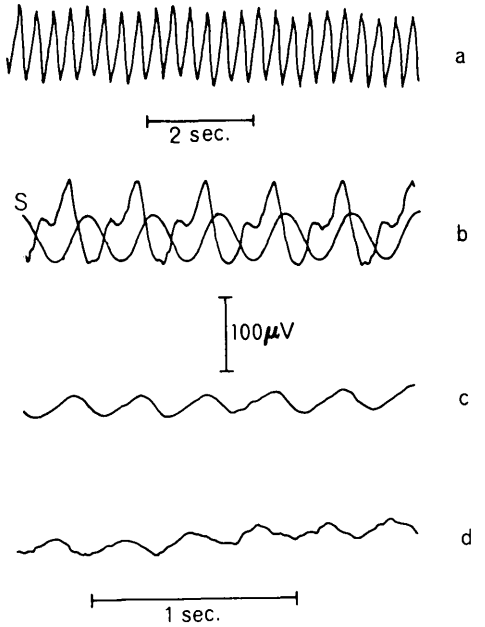

Fig. 4. All waveforms are the response to a 3 Hz. sinusoidal input. A typical record is shown in *a*. This type of record is found at the beginning and end of an experiment in which the frog survives to be used again. Atypical records are shown in *b*, *c*, and *d* and are found only when degeneration of the retina progressively takes place during the course of an experiment inferred from the animal's death. Demonstrated in *b* is the changing harmonic content of the response two hours after the experiment started, while *c* indicates a stage 1½ hours later showing a change in both harmonic content and amplitude. In *d*, a further stage is shown, in which after *c* the eye is dark adapted for about ½ hour and the response recorded. The vertical calibration is for the atypical records shown in *b*, *c*, and *d*.

the very low frequency range from 0.1 Hz. down to 0.07 Hz. These waveforms demonstrate the occurrence of higher harmonic terms in the output in the very low frequency range. This is not always seen in our results, but when it does occur it is quite consistent throughout the experiment.

The gain-frequency characteristic exhibits a dip or a trough at intermediate and low frequencies. This is seen in Fig. 4 for the input-output data of the system. In these waveforms the amplitude falls and then rises again with decreasing frequency. This increasing response amplitude can be observed in Fig. 2 in which

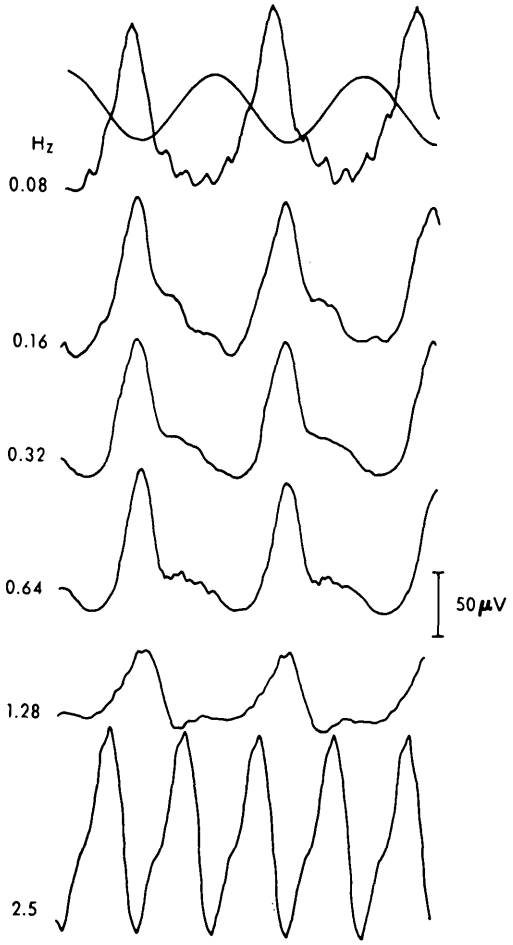

Fig. 5. Frequency response from 0.08 to 2.5 Hz. The records have been normalized such that amplitudes can be compared. Note that the amplitude decreases from 2.5 to 1.28 Hz. and then increases with decreasing frequency. These records are measured at the corneal surface.

the amplitude doubles over about a decade. Occasionally, it is observed that the frequency at which maximum harmonic distortion occurs shifts compared to the normal or typical response. This change when it is observed takes place over several hours, usually from the beginning to the end of a single experiment. This is demonstrated in Fig. 5. These changes are most pronounced in animals that do not survive. Animals that survive to be used again show the typical type of response. Most of our recordings show a low amplitude ripple especially at very low frequencies. The ripple frequency is of the order of one per second. With retinal degeneration inferred from the onset of death, the ripple appears to reduce in amplitude.

Discussion

The very low frequency characteristics of visual systems are difficult to obtain. In this paper, we have described preliminary attempts to obtain these very low frequency responses of the frog retina, when the intact eye is sinusoidally stimulated with light. These experiments have lead to some rather tentative conclusions regarding the bandwidth and phase characteristics of the system. First the bandwidth of the system appears wider than we had previously thought, the system exhibiting substantial gain at frequencies as low as 0.01 Hz. For example, the gain roughly doubles over about a decade from 0.1 Hz. to 0.01 Hz. (Fig. 2). A dip also appears in the low frequency gain characteristics. That is, the gain falls and then rises again. This implies an increasing retinal sensitivity in the very low frequency range. It is not too improbable that a reduction in stimulus frequency in this range enhances the rod response. Furthermore at very low frequencies there is a rather substantial phase lag between sinusoidal input and the system output. This is clearly shown in Fig. 2 in which, at 0.07 Hz., the input and output are essentially out of phase. Also, the turnover in the ERG waveform is much more rapid when the light passes through its minimum intensity than when it passes through its maximum intensity.

The observation that changes in harmonic distortion occur with a frequency shift at which maximum harmonic distortion takes place is most probably related to metabolic factors. This possibility has also been pointed out by Troelstra.[20] The low amplitude ripple seen clearly at very low frequencies most probably represents a cardiovascular component. This is substantiated by the fact that the ripple at-

tenuates with retinal degeneration, assumed to take place with the onset of the animal's death.

An important consideration is whether the low frequency data presented contain any EOG component. This will now be considered. The amplitude of the EOG is a function of the level of retinal adaptation.[21, 22] This resting potential falls to a minimum during dark adaptation. During light adaptation it reaches a maximum and then decreases to the preadaptation state. The time course to reach minimum from the light-adapted stage is of the order of six minutes. To reach maximum starting from the dark-adapted state takes approximately the same time interval. If we consider time rates of change of this potential, then the maximum rate of change of the EOG occurs when the light level is abruptly changed, as in the case of a step input from light on to lights off. Under these circumstances the time rate of change of the EOG is of the order of 10 to 20 μv per minute in the rabbit[22] and about 30 μv per minute in the human being.[23] In both cases the recording electrodes were arranged in the horizontal plane on either side of the eye. In the case of the rabbit the EOG as a function of the adaptional state was measured periodically by mechanically rotating the eye.[22] In the human investigation, the eye was stimulated in a sinusoidal fashion utilizing periods in the range of about 20 to 30 minutes. Eye movements were elicited every ten seconds to obtain the EOG. The natural frequency of the EOG system corresponds to a period of about 25 minutes.[24] This frequency is about 15 times slower than the lowest frequency used in this study.

In our stimulus-response data the lowest stimulus input frequency completes a full cycle in 100 seconds. At this lowest stimulus frequency the EOG would have a maximal influence on our data. The response to a sinusoidal stimulus of 0.01 Hz. has by a straight line approximation a maximum rate of change of about 20 μv per second. Hence the EOG and our retinal response data differ by a factor of about sixty in their time rate of change. Likewise, the phase difference between stimulus and response in the data presented appears incompatible with EOG changes. At certain low frequencies the stimulus and response waveforms are completely out of phase. If the light input is producing a change in the adaptational state resulting in a change of EOG, then a decreasing stimulus would give rise to a decreasing EOG. When triangular stimulus waveforms are employed, the point is more cogently demonstrated. The maximum retinal response occurs when the light passes through zero and therefore can not be the EOG.

We conclude then that we are measuring the steady-state ERG at all frequencies. Slow changes in the stimulus intensity most probably cause some shift in the EOG. Differences in the dynamics of the two systems (EOG and ERG) indicate a minimal influence of the EOG on the data presented in this paper. Rod enhancement with cone suppression probably accounts for the increased retinal sensitivity observed at very low frequency.

The frog electroretinographic system is not just a simple band-pass system since it exhibits rather complex changes in harmonic content as a function of input frequency. These changes give rise to a linear-nonlinear-linear (L→N→L) transition as a function of frequency. Some of our experiments suggest that the system is even more complex, the transitions being L→N→L→N→L as a function of frequency. However, this point will have to be pursued further.

The typical transition L→N→L seen in the frequency domain can be explained on the basis of two cell populations. Troelstra[16] has shown that such transitions can be accounted for by considering the system exhibiting two separate delays with a fixed interdelay time. Theoretically, it can be acounted for by a cascaded system of linear-nonlinear operators with the final

linear element exhibiting direct current gain but having a notch in its low frequency gain characteristic.[15, 17, 19, 25]

The main component of the ERG is the b-wave,[26] and it is assumed that in the steady-state sinusoidal response the b-wave–generating system makes the biggest contribution to the output. Miller and Dowling[29] have recently demonstrated that the b-wave generator is the Müller cell in the mudpuppy. If the two subsystem hypothesis is correct, then it has to be assumed that two types of cells exist having different characteristics. This would imply two types of Müller cells. Let us assume the existence of these two systems each, providing its own response or effect. We can also label these two effects, A and B, without specifying their function and assume that at very low frequencies only A effects are observed, at intermediate frequencies both A and B effects are observed, and at higher frequencies only B-effects are observed for sinusoidal inputs. We can now question their relative contributions to the steady-state ERG as a function of frequency. Under these circumstances the very low frequency characteristics would be A-type characteristics, while the high frequency characteristics would be B type. The intermediate frequency characteristics would be a mixture of both A and B types, and the intermediate frequency would then contain both A and B effects. This explanation would be reasonable in terms of the linear-nonlinear-linear transition observed in the frequency domain. The combined effects of A and B at intermediate frequencies would produce the good intermediate characteristics and the harmonic distortion seen in the frog ERG. This would imply a frequency at which the harmonic content is maximum at all depths of modulation flanked by frequency ranges in which the harmonic distortion continuously diminishes with either decreasing or increasing frequency. The adjacent frequency bands (very low and high) would represent A and B alone and their mode of low and high frequency transmission, respectively. It would further imply the existence of two retinal networks processing information in a parallel fashion but having different transfer functions. The system defined by A most probably incorporates the rod photoreceptors, while system B incorporates the cones. The electrode sampling the total population response would then monitor system A or B or both depending on the input frequency.

Such results would be in keeping with the demonstration that no improvement in linearity results at intermediate frequencies by decreasing the depth of modulation and likewise the higher frequency side band would be approximately sinusoidal at all modulation depths.[19] A similar result should occur at the low frequencies. Experimentally, this is the case as demonstrated in Fig. 2.

Byzov[27] has argued for two such bipolar systems, both operative at low frequencies corresponding to our intermediate frequency range and only one operative at higher frequencies corresponding to our high frequency range. Granit[28] has likewise postulated two retinal systems competing for the same set of central connections with one becoming dominant by inhibiting the other at higher frequencies.

In keeping with this hypothesis, should the retina degenerate, it is conceivable that systems A and B will decay at differing rates. Theoretically, the harmonic distortion at a particular frequency in the intermediate range could change and simultaneously the amplitude of the response diminish. This implies that the relative contributions of system A and B change as a function of frequency. The data presented in Fig. 5 lend themselves to this hypothesis. However, since retinal degeneration involves the state of retinal metabolism such a hypothesis would have to be carefully evaluated from metabolic considerations. Such considerations would necessitate experimentation to measure retinal metabolism and the identification

anatomically and functionally of systems A and B.

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