The resonant frequencies of rod and cone electroretinograms

Peter Gouras and Ralph D. Gunkel

The ERG of the albino rat and the gray squirrel was studied with sinusoidally flickering light and D.C. amplification. It was found that: The rat's ERG has a resonant frequency of 2 to 4 c.p.s. with attenuation at both lower and higher frequencies; the bandwidth of the response extends from 0.05 to 25 c.p.s. The squirrel's ERG has a resonant frequency of 9 to 10 c.p.s. with a bandwidth of approximately 1 to 100 c.p.s. Increasing the light intensity broadens the bandwidth but does not shift the resonant frequency greatly. Analogies are made between the response of these pure rod and cone retinas and the low-frequency characteristics of human vision.

Psychophysical studies have revealed that the visual system of the human being has a frequency response which attenuates low- as well as high-frequency light stimulation. The scotopic system is apparently tuned to a frequency of about 4 cycles per second (c.p.s.) and the photopic system to the higher frequency of 8 to 10 c.p.s. The location of this filtering action within the visual system is not known. The fact that the eye is more sensitive to transient than to steady illumination is indicated by the results obtained with the Ganzfeld technique, and the records extant from vertebrate optic nerve fibers. It would appear important to determine how much steady-state information, if any, is conveyed to the optic nerve and where the low-frequency attenuation occurs. The stabilized image and Ganzfeld techniques are limited by both their subjective nature and the difficulties in obviating ocular movements and the recordings from optic nerve fibers by their inherent selectivity. The electroretinogram (ERG) reflects the mass activity of cells more peripheral than the optic nerve and is intimately related to the visual act, for an extinguished ERG indicates absent vision. Its frequency response should reveal the filtering action of a very peripheral electrical event in vision.

To determine its frequency characteristics, sinusoidally flickering light has been used to elicit ERG responses from the predominantly rod eye of the rat and the pure cone eye of the squirrel. It was considered that the frequency response of these ERGs might reflect the two characteristic types of retinal responses, scotopic and photopic, which exist side by side in the human eye. The correlation between the electrophysiologic responses obtained in this study and the psychophysical reports support this assumption and indicate that the low-frequency filtering in the human visual system may be predominantly retinal in origin.

Methods

An adult albino rat (Osborn-Mendel strain) and a gray squirrel (Sciurus leucotis carolinensis)
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were studied. The animals were anesthetized by an intraperitoneal injection of Nembutal (35 mg per kilogram). In addition, corneal anesthesia was provided by topical application of 0.5 per cent proparacaine hydrochloride (Ophthaine) and a drop of 0.5 per cent methylcellulose applied to the conjunctival surface to facilitate electrical contact. Care was taken to carry the anesthetic level deep enough to eliminate eye movements which complicate accurate D.C. recording. Van der Tweel and Visser found no difference in the responses of cats to sinusoidal light whether under barbiturate anesthesia or in "encephale isolé." The ERG was recorded differentially between an Ag-Ag Cl electrode in a methyl methacrylate scleral contact lens and a stainless steel needle electrode placed under the skin on the forehead. The potentials were recorded by a Model 5 chopper-type direct-coupled amplifier and ink writer* with a high-frequency response showing 50 per cent attenuation at 60 c.p.s.

The light source was a 1,000 watt high-pressure xenon arc lamp which was collimated and brought to a focus at the animal's pupil by a lens subtending approximately 60° of visual field. It produced a corneal illumination of approximately 7,500 lux, which could be varied by neutral density filters. A small portion of the beam was reflected to a selenium photocell which monitored the frequency of the sinusoidally flickering light. Sinusoidal light was obtained by means of a plastic disc covered with a series of opaque sectors extending radially from the hub and symmetrically around the disc so that the relatively transparent periphery became gradually more dense toward the center. The disc was mounted on miniature ball bearings and rotated at high speed (500 to 1,000 c.p.s.) by an air jet directed at its serrated edge. The rotating disc was driven to and fro across the light beam at a focal point by a variable low-speed motor, thereby changing the light intensity in a slow sinusoidal manner. This was used both directly for stimulating the rod eye at very low frequencies and also for producing a circular sinusoidal wedge which was made by exposing a disc of low-contrast film by rotating it synchronously in the path of the sinusoidal beam. This wedge could be rotated at much higher speeds than the previous device; it produced a sinusoidal modulation of 65 per cent, whereas that obtained by the rotating disc was about 75 per cent. The closeness of the overlap between these two methods is shown by the curve for the rat in Fig. 1 at 2 c.p.s. where the ERG amplitude obtained by both methods is plotted. The sinusoidal shape of the pulses can be seen in the lower traces of A, B, C, and D of Fig. 2 which is a D.C. coupled recording of the output of the monitoring photocell.

The responses to the sinusoidally varying light were obtained for only a few seconds at any one frequency and the whole process was repeated in both directions through the maximum in order to detect any changes resulting from light adaptation.

*Grass Instrument Company, Quincy, Mass.
Results

The results of this study are shown graphically in Fig. 1. The ERG of the rat has a resonant frequency of approximately 2 to 4 c.p.s. The bandwidth extends from at least 0.05 to 25 c.p.s. The resonant frequency of the squirrel's ERG is much higher at approximately 9 to 10 c.p.s. and has a bandwidth extending from approximately 1 c.p.s. to at least 100 c.p.s. Increasing the light intensity increases the amplitude of the response and widens the bandwidth but has only a slight effect on the resonant frequency. There is only a shift of the peak from 9 to 10 c.p.s. with a 2.1 log unit increase in light intensity in the case of the squirrel. Prolonged dark adaptation shifts the curve of the rat along the ordinate axis without changing its shape and had little effect on that of the squirrel. The maxima were also independent of the direction in which the frequency was changed, indicating that light adaptation during the testing period was insignificant. The greater amplitude of the responses obtained from the squirrel appears to be related more to an inherently greater electromotive force produced by its retina than to its rapid dark adaptation.

Fig. 2 shows some of the ink-writer records of the ERG responses obtained at different frequencies of sinusoidal stimulation of both animals. At very low frequencies, the rat's ERG (Fig. 2, A) closely follows the wave form of the stimulus, whereas no ERG response can be detected from the squirrel (Fig. 2, E). At slightly higher frequencies, an ERG is detectable from the eyes of either species but with quite different characteristics. The rat eye follows the wave form of the stimulus (Fig. 2, B), whereas there is a marked phase shift in the response of the squirrel's eye (Fig. 2, F), demonstrating its sensitivity to the time derivative and not the magnitude of the stimulus. As the flicker rate increases the amplitude of the squirrel's ERG (Fig. 2, C) grows rapidly, whereas the rat's ERG (Fig. 2, D) begins to diminish. It is noteworthy that the phase shift apparent at low frequencies in the case of the cone ERG decreases as the frequency of maximum response is approached. The D.C. recording shows a basic difference in these two types of responses. In the vicinity of the resonant frequency, the squirrel's ERG oscillates above and below the steady corneal potential, whereas the rat's ERG is characterized by a series of positive responses to the light. With more prolonged illumination (5 to 10 seconds), the entire D.C. level slowly begins to shift negative in both these animals. This is greater in the rat and can be seen even with the relatively short durations of stimulation in Fig. 2, C, D. The rat's ERG also shows a lag in following the first few stimuli of a rapidly flickering light, shown in Fig. 2, D where the first few responses are just discernible riding on the b-wave responses to the fast transient of the light pulse. In addition to the positive off-responses, which are very large in the case of the squirrel (Fig. 2, G), D.C. recording reveals a slow negative off-response in the rat's eye (Fig. 2, D) which takes as long as 5 to 10 seconds before it returns to the base line. The conventional use of condenser-coupled amplification systems have undoubtedly hitherto made it difficult to record this response from the rat's eye.

Discussion

That the ERG of cone retinas has a much higher flicker-fusion frequency than that of rod retinas is known, but the frequency of maximum response, as well as the low frequency characteristics of these distinctly different ERGs, has never been determined. It is only by the use of sinusoidal light that one is able to demonstrate that the ERG of a cone retina has a much higher resonant frequency but a much steeper low-frequency attenuation than that of a rod retina. Thus, it may be possible by the use of low-frequency sinusoidal light not only to enhance the rod response but actually to suppress the cone response of mixed retinas in a way that is analogous to the uncovering of cone-flicker response from...
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predominantly rod retinas by the use of high-frequency flicker rates. Although it is difficult to compare the electrical responses of rod and cone retinas of animals with subjective measurements, the close correlation between the high sensitivity of the human visual system to flicker at 4 c.p.s. scotopically and 8 to 10 c.p.s. photopically and the resonant frequencies of the rod and cone ERGs suggest that similar mechanisms may be involved. There is, however, a much more marked low-frequency attenuation in the ERG responses than in the psychophysical studies of either Ives or de Lange. The ERG curves are, of course, amplitude measurements, whereas the psychophysical studies involve thresholds. Amplitude measurements do not necessarily parallel those of thresholds especially when the response amplitudes are large. The fact, however, that the ERG responses are not only markedly diminished but undetectable at low frequencies indicates that sensitivity must also be reduced particularly in the case of cone activity. There is evidence that such human psychophysical curves for photopic vision also exhibit a more pronounced low-frequency attenuation when the effects of eye movements are minimized.

Recently Van der Tweel and Visser have also employed sinusoidally modulated light to elicit ERG responses from cats and humans. The cat shows a maximum response to modulation of 25 per cent at 0.5 c.p.s., the lowest frequency reported in this study, and a secondary maximum at 6 c.p.s. To the same extent there is a “kink” in their phase-shift curves at 6 c.p.s. It is interesting to speculate on whether these changes represent the interaction of relatively independent rod and cone activities of this mixed retina which contribute differently to the mass response of the ERG at different frequencies. The frequency of maximum response of the cat's ERG is extremely low and the frequency-response curve demonstrates no low-frequency attenuation despite the fact that D.C. recording was not used. It would be important to know whether there is, in fact, any low-frequency attenuation in this animal or whether the ERG follows steady changes in the light intensity.

The low-frequency attenuation of the ERG shown by this study may be important in considering the dynamic nature of vision. Recent studies with stabilized retinal images reveal that information about steady-state photochemical events in the photoreceptors tends to be lost somewhere in the visual system when the effects of eye movements are eliminated. The same is undoubtedly the case for results obtained with the Ganzfeld technique which eliminates scanning effects by using a diffusing sphere to carry the image of a homogeneous field to the limit of functioning retina. The high sensitivity of the ERG to the time derivative and not the absolute level of light intensity suggests that little electrical activity is generated in the retina to stationary fields, especially in the case of photopic or cone vision. It has recently been shown that there is a linear relationship between the amount of functioning retina and the ERG sensitivity, suggesting that an absent ERG should indicate absent vision. To the same extent the absence of an electrical change at the cornea during light stimulation at least 5 log units above ERG threshold (Fig. 2, E) suggests that the animal may be receiving little effect of this stimulus in its more central nervous system.

There is much support for the fact that the optic nerve carries little information about steady-state phenomena from the majority of optic nerve fiber responses studied so far. There are, however, reports that some vertebrate neurons discharge continuously to a steady light stimulus and there are also potentials recordable from within the retina near where the ERG is generated which are maintained to continuous photic stimulation. This indicates that either there may be little relationship between the ERG and the optic nerve message or the more reasonable hypothesis...
that the electrical changes produced by steady-state phenomena are relatively small and undetectable by the current sensitivity for recording the ERG. Small shifts in the D.C. potential are found at the cornea in both the rat and the squirrel eye to maintained illumination and may be the counterpart of what steady-state activity occurs within the retina.

The use of the time derivative of a stimulus at this peripheral level in vision has certain advantages in communicating information from the retina to the optic nerve since only change would be transmitted, a very economical use of a limited number of communication lines. That movements of the eye may serve to enhance vision would implicate the extraocular muscles closely in a feedback loop to the retina. By controlling the frequency at which an image traverses the retina, eye movements can either enhance or attenuate the electrical signal and thereby the visual sensation. The power spectrum of extraocular muscle movements has not been measured, but it would be interesting to know how it parallels that of the ERG in both photopic and scotopic conditions.

REFERENCES