Frequency Characteristics of Retinal Neurons in the Carp

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ABSTRACT Frequency characteristics of various retinal neurons in the carp were studied using sinusoidally modulated light as an input. They were affected by both intensity and pattern of illumination. In the horizontal cells, in which the effect of light intensity was studied most extensively, an increase in the light intensity brought about a decrease of the gain, which was more marked at lower frequencies, resulting in a shift of cutoff frequency towards higher frequencies and in a slight low frequency attenuation. A decrease in the area illuminated had an effect similar to a decrease in the light intensity. In the receptor, the low frequency attenuation was not apparent even at high light intensities. The adaptation process in receptors was not sufficient to explain the low frequency attenuation in the horizontal cells, and a possible contribution of negative feedback from horizontal cells to receptors was suggested. In the bipolar cell, the lateral interaction played an important role. An increase in an area resulted in the suppression of the response at low frequencies where the phases of the center and the surround responses were opposed, but in the augmentation near 5 Hz where the two responses were in phase. In amacrine cells, a low frequency attenuation and a phase advance at low frequencies were very prominent, and were considered to be due mainly to a process designated here as the neural adaptation.

INTRODUCTION

Sinusoidally modulated light stimuli are now widely used in neurophysiological studies of both vertebrate and invertebrate visual system (see Cleland and Enroth-Cugell, 1966 for the earlier works on this topic). These studies have shown that for small changes of the light intensity the visual system can be treated as a linear system. In the vertebrate, this kind of experiments have been mostly confined to the ganglion cells (Cleland and Enroth-Cugell, 1966; Hughes and Maffei, 1966; Spekreijse, 1969; Maffei et al., 1970; Schellart and Spekreijse, 1972), except for one report on the S-potential from horizontal cells in the goldfish retina (Spekreijse and Norton, 1970). The frequency

characteristics of the ganglion cell responses depend on the stimulus area and also on the level of adaptation. An increase in the area results in an attenuation of the response at low frequencies and an enhancement at a certain high frequency range (Maffei et al., 1970). A similar effect has been observed in the human flicker threshold (Kelly, 1969; Fiorentini and Maffei, 1970) and in the response of the eccentric cell of *Limulus* (Ratliff et al., 1967). In all these studies, the effect has been ascribed to lateral inhibition. An increase in the mean light intensity results in an attenuation of the ganglion cell response especially at low frequencies (Schellart and Spekreijse, 1972) as also was observed in human flicker experiments (deLange, 1958; Kelly, 1961; Roufs, 1972). Because of this low frequency attenuation, the gain characteristics of the response exhibit a peak at a certain frequency, which becomes more prominent with further increase in the light intensity. Although no explanation for the mechanisms underlying this low frequency attenuation has been proposed in the vertebrate visual system, a similar attenuation observed in the visual cells of *Limulus* has been considered to reflect a kind of adaptation process (Dodge et al., 1968).

It is probable that some kinds of adaptation processes and other processes such as feedback and lateral interaction among neurons play an important role in modifying the frequency characteristics of the vertebrate retinal neurons. For the analysis of these processes which could take place at levels distal to the ganglion cells and would determine the dynamics of the response, it is necessary to study the frequency characteristics (transfer functions) of individual neurons constituting the complex neuronal chain from receptors to ganglion cells. In the present experiments, the responses of receptors, horizontal cells, bipolar cells, and amacrine cells are recorded intracellularly in the carp retina. Analysis of their frequency characteristics discloses various types of interaction among neurons at different stages of the retinal transmission as will be described below.

METHODS

Intracellular Recording

Carp (*Cyprinus carpio*) of 500–600 g were used in the experiments. They were kept in darkness for more than 1 h before excision of the eye. The isolation of the retina from the pigment epithelium was easier after the dark-adaptation. The isolated retina was placed receptor side up in a small chamber with its bottom made of glass. Micropipette electrodes filled with 2.5 M KCl solution were advanced vertically from above, and the penetration into single cells was aided by jolting the preparation (Tomita et al., 1967). The resistance of the pipette was in the range 50–150 MΩ in Ringer's solution.

The retina was illuminated once every 4 s with white light of 0.6 s duration from underneath. A light spot of variable size and an annulus preadjusted to 0.8 mm inner

and 4 mm outer diameter, either of white light or of monochromatic light, were available from the two-channeled photostimulator (Tomita et al., 1967). Whenever a response was recorded intracellularly, its spectral sensitivity and receptive field organization were first examined in order to identify the cell type. Several criteria for the identification of cell types have been already reported (Tomita et al., 1967; Kaneko, 1970; Toyoda, 1973; Toyoda et al. 1973).

It has been shown by histological studies of Stell (1967) that external horizontal cells and small bipolar cells of the goldfish retina receive synaptic inputs exclusively from cones, and intermediate horizontal cells from rods. These observations were confirmed by physiological studies of Kaneko (1970) and Kaneko and Yamada (1972) who also provided evidence that the internal horizontal cells receive inputs from cones. Large bipolar cells, on the other hand, receive synaptic inputs from both rods and cones (Stell, 1967). In the present study, the second order neurons receiving predominant inputs from rods, such as intermediate horizontal cells and large bipolar cells, were left out of the results because it was afraid that analyses of the responses would be more complex if the rod system possessed frequency characteristics quite different from those of the cone system. In the study of amacrine cells, which all receive inputs from large bipolar cells and consequently receive indirect inputs from both rods and cones, their response was recorded only after the retina was light adapted by preadapting light to avoid the contribution from the rod system.

Measurement of Frequency Characteristics

The sinusoidally modulated light was obtained from a glow modulator tube (Sylvania 1131C; Sylvania Electric Products, Inc., New York) by frequency modulation of the driving pulses. The duration of each pulse was usually set to 1.0 ms. The mean frequency of the pulse train was about 400 pulses/s and the depth of modulation (½ peak to peak) was either 30 or 40 % of the mean. The light intensity was controlled by neutral density filters inserted in front of the tube. The light was then guided through a fiber-optics bundle and illuminated the retina diffusely from above.

Responses amplified through a DC amplifier and displayed on an oscilloscope were continuously photographed on moving film. Pulses synchronous to the trough of the stimulus sine wave were fed to the z-axis of the oscilloscope to brighten the trace and thus put marks on the record for later calculation of the phase relation. Sample records of a horizontal, a bipolar, and an amacrine cell response are shown in Fig. 1.

Linearity

At the beginning of the present experiment and later, only when necessary, the linearity of the response was monitored by applying input and output signals simultaneously to the vertical and the horizontal axis of the oscilloscope so as to produce a Lissajous figure. The range of linear response depends upon the mean light intensity and upon the amplitude and frequency of modulation. For frequencies over 1 Hz, the response amplitude increased linearly with increase in the stimulus amplitude up to about 80 % depth modulation, the maximum modulation available in the present system. At a low frequency range, however, a slight nonlinearity, though only at high
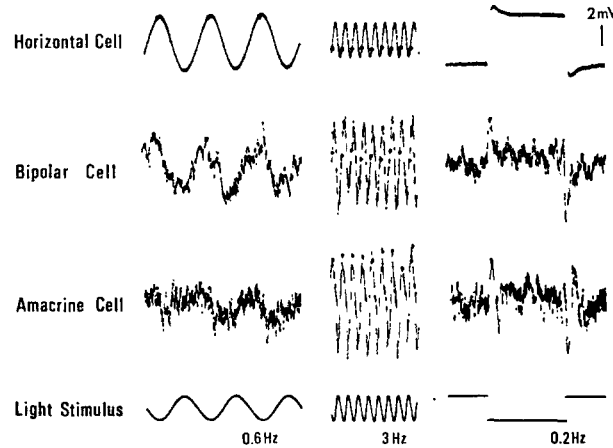
FIGURE 1 The response of an external horizontal cell, a bipolar cell ("off center type), and an amacrine cell ("off" type) to small sinusoidal and square-wave modulations of the light intensity. Modulation frequencies in this figure are 0.6 and 3 Hz for the sine wave and the duration of the light decrease is 2.5 s for the square wave. The depth of modulation ($\frac{1}{2}$ peak to peak) is 30% of the mean. Small spots on the trace marked by brightening indicate the trough of the input sine wave and formed the reference points for the calculation of phase relationships. The light stimulus at the standard intensity used in these records is diffuse light from a glow modulator tube, and is about 2.0 log units above threshold for the response of external horizontal cells.

light intensities, was observed in the Lissajous pattern even with a modulation depth of less than 40%.

In order to compare the frequency characteristics of various retinal neurons at the same mean light intensity, it was necessary to define the standard light level. The light intensity giving rise to a barely noticeable deflection (less than 0.1 mV) of the photopic luminosity type S-potential was defined as the threshold. The standard light level was set to 2.0 log units above this threshold, and was equivalent in its effect on the luminosity type S-potentials to monochromatic light of about 1.5 $\times$ 10^3 quanta $\cdot \mu m^{-2} \cdot s^{-1}$ at 620 nm. All other intensities used are expressed in log units relative to the standard. At this standard light intensity, the S-potentials from horizontal cells were almost linear throughout the frequency range studied. The bipolar and amacrine cell responses, especially the latter, on the other hand, showed a slight nonlinearity at low frequencies. The gain and phase characteristics were expressed on Bode plots, where both frequency of the input sine wave and the amplitude of the sinusoidal response were plotted on logarithmic scales. The relative amplitude (R) was expressed in decibels in all subsequent figures according to the equation $R = 20 \log (A_f/A_{fo})$dB. A_f is the amplitude of the sinusoidal response at a modulation frequency f. A_{fo} is the reference amplitude, usually at an extremely low frequency. When it was necessary to compare the relative amplitudes of different sets of data, as in Figs. 3 and 6, A_{fo} from one set of data was used as a common reference point.

RESULTS

Receptors

The response of carp cones recorded intracellularly is hyperpolarizing to illumination (Tomita, 1965; Tomita et al., 1967). It can be identified from criteria such as the depth of recording, the area effect, and so forth (Kaneko and Hashimoto, 1967). The responses recorded in the present experiments were small and generally did not last long enough to permit an analysis of their frequency characteristics. The data marked by filled circles in Fig. 2 are from an exceptionally stable unit, which have a response large enough to plot several data points. The frequency characteristics of a horizontal cell under the same condition, taken from Fig. 3, are also plotted with a broken line in Fig. 2. At this light intensity level, the characteristics of the cone response resembled those of horizontal cells, except for less attenuation in cones at higher frequencies.

The cone response becomes faster in its time course with an increase in the background light intensity. This is demonstrated by data points marked with open circles in Fig. 2, which were obtained in another unit at the mean light intensity 2.0 log units higher than the standard. The frequency range covered by these data is not wide enough, but no other records stable enough to cover different frequency ranges were available. In some experiments, therefore, the mass receptor potential (distal PIII) isolated from the rest of the electroretinogram (ERG) components by means of a coaxial electrode (Murakami and Kaneko, 1966) was studied instead. The data plotted with open squares in Fig. 2 are those of the distal PIII at the mean light intensity 2.0 log units higher than the standard. The contribution of the rod activity was considered negligible at this light intensity because the spectral response curve of the distal PIII under the same adaptation level was similar to that of the red cones in the carp retina (Tomita et al., 1967). There is a fairly good agreement between the gain characteristic of the receptor potential recorded intracellularly and that of the distal PIII. The gain characteristic of the distal PIII is almost flat at lower frequencies, and contrasts with those of the S-potential under the same illumination condition, shown in Fig. 3 and also retraced in Fig. 2 with a dotted line for comparison. Thus the carp cones behave differently from the invertebrate photoreceptors which show a marked low frequency attenuation at high light intensities (Pinter, 1966; Zettler, 1969; Knight et al., 1970).

In these experiments on the distal PIII, it was difficult to rule out a possible contamination of the response by other components of the ERG such as the PII and the proximal PIII originating at the retinal layers other than receptors. This was also true for the intracellular recordings, since the receptor
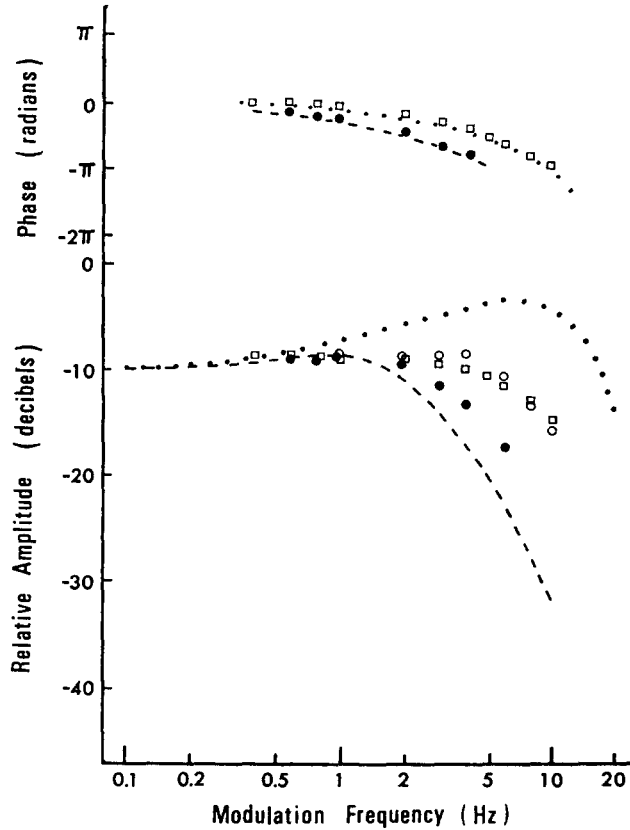
FIGURE 2 The frequency characteristics of receptors (cones). Filled circles: data from a cone response recorded intracellularly at the standard light intensity. The frequency characteristics are similar to those of the S-potential (shown with a broken line) except for less attenuation of the gain in cones at high frequencies. Open circles: relative amplitudes of another cone response at an intensity 2.0 log units higher than the standard. Open squares: data from the distal PIII (the mass receptor potential isolated by means of a coaxial electrode from the rest of the ERG components) at an intensity 2.0 log units higher than the standard. The low frequency attenuation observed in the S-potential at the same intensity (shown with a dotted line) is not apparent in the cone and in the distal PIII.

potential was not more than five times larger than the ERG. It was also attempted in some experiments to isolate the receptor potential by application of chemicals such as sodium aspartate to block all the responses of the second order and other higher order neurons (Cervetto and MacNichol, 1972; Murakami et al., 1972). The frequency characteristics of the mass receptor potential isolated by sodium aspartate showed a high frequency cutoff starting at a frequency slightly lower than that of the distal PIII without aspartate. This means that the response becomes slower after aspartate. It is possible

that the slowing of the receptor potential after aspartate is physiological, because the potential is free from the contamination and from the interaction by the responses of higher order neurons. On the other hand, it is difficult at present to rule out the possibility that such chemicals affect the time course of the photic responses of receptors themselves. For this reason, no further studies were made with such chemicals.

Horizontal Cells

There are three types of horizontal cells in the teleost fish: external, intermediate, and internal (Cajal, 1892). Both histological and physiological studies have shown that the intermediate horizontal cells receive inputs exclusively from rods (Stell, 1967; Kaneko and Yamada, 1972). The frequency characteristics of intermediate horizontal cells, thus representing the rod system, will be described not in this paper but in a separate paper (Toyoda, J., in preparation). The S-potential from external as well as internal horizontal cells can be classified, according to their spectral responses, into the luminosity type (L-type) and the chromaticity type (C-type) (Kaneko, 1970). In the present experiments, only L-type S-potentials were studied. The responses from the external and internal horizontal cells can be distinguished by careful examination of the depth of recording and the size of the receptive field (Kaneko, 1970). The frequency characteristics of the two, however, were not apparently different.

In each experiment, the frequency was scanned first from 1 Hz and up, and then from 1 Hz and down. Control records at 1 Hz and at several other frequencies, when necessary, were repeated after recording a response to the square wave modulation at 0.2 Hz. Records showing more than 5% change in the response amplitude at 1 Hz were discarded. Reliable data were obtained from 32 horizontal cells. Two of them were stable enough to cover the intensity range of 4 log units, and five of them 3 log units.

Except for three units, which were recorded in the same preparation, the frequency characteristic curves of S-potentials at the same intensity level overlapped with each other within a range of ±3 dB. Especially, all units from the same preparation showed almost identical frequency characteristics. This finding was important because it allowed a comparison of the frequency characteristics of different neurons, such as horizontal cells and bipolar cells, provided that both neurons are recorded from the same preparation. Whenever the data from a bipolar or amacrine cell were obtained, the frequency characteristics of the S-potential in the same preparation was studied as a control.

The frequency characteristics of the horizontal cell to diffuse light at different mean light intensities are shown in the Bode plots of Fig. 3. At low light intensities, the gain characteristics of the S-potential are almost flat at a low
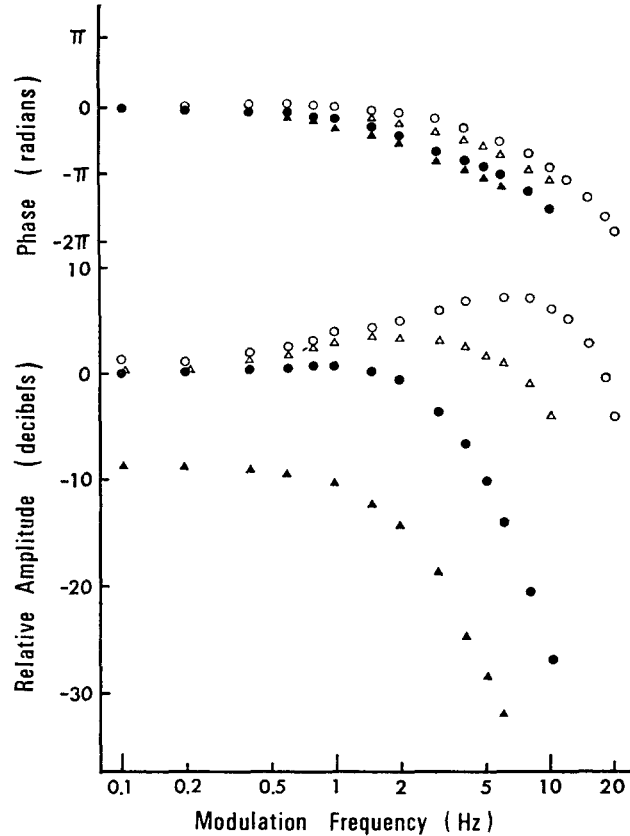
FIGURE 3 Gain and phase characteristics of a S-potential at different mean light intensities. Filled triangles: data for an intensity 1.0 log unit lower than the standard. Filled circles: those for the standard intensity. Open triangles and open circles: those for intensities 1.0 and 2.0 log units higher than the standard, all under diffuse illumination. The response amplitude at an extremely low frequency under the standard light intensity was set to 0 dB. All other amplitudes are plotted relative to this value irrespective of the difference in the mean light intensity. The percentage of modulation, however, was held constant. The amplitude of the response at a low frequency range stays almost constant for a fairly wide range of intensities as long as the percentage of modulation is held constant. With an increase in the mean light intensity, the cutoff frequency becomes higher, and a resonant peak due to a low frequency attenuation becomes prominent.

frequency range, and show a steep cutoff toward higher frequencies. In order to compare the relative amplitude of the response at different light intensities, the amplitude of the sinusoidal response to 0.1 Hz at the standard light intensity was taken as the reference point (A_{fo}). It is seen in this figure that the amplitude of the response to 0.1 Hz stays almost constant for different light intensities above the standard. Since the percentage of modulation is fixed for all these records, an increase in the mean light intensity implies an

increase in the amplitude of modulation. It follows from these results that the sensitivity of the horizontal cells to extremely low frequency stimuli decreases 10-fold with each 10-fold increase in the mean light intensity, satisfying the Weber-Fechner logarithmic law. At extremely low intensities, for instance, at about 1.0 log unit below the standard as shown with filled triangles in Fig. 3, the records deviate from this logarithmic relation. Thus, with an increase in the light intensity, the sensitivity of the response is markedly reduced. But this sensitivity decrease is less marked at higher frequencies so that there appears a peak in the gain characteristics at a certain intermediate frequency. The peak becomes more pronounced and shifts towards higher frequency if the mean light intensity is further increased. In other words, the S-potential becomes more rapid in its time course at higher background light intensity.

The phase characteristics of the response are also plotted in Fig. 3. At a low light intensity, there is no phase lead but a phase lag which increases towards higher frequencies. At high light intensities, a phase lead, although often barely noticeable, is seen in a low frequency range.

Effect of stimulus area on the S-potential

The effect of area illuminated on the frequency response of horizontal cells was studied in the following two ways. In one of these, a steady annular background light of 0.8 mm inner diameter at various intensities was applied. The data shown with open triangles in Fig. 4 are control records with a diffuse test light at an intensity 1.0 log unit above the standard. The presence of a steady annulus at an intensity almost equal to the diffuse test light did not affect the gain characteristics of the S-potential appreciably. However, the steady annulus at an intensity 10 times the test light augmented the response at high frequencies as shown with open squares in Fig. 4. A part of this effect could come from the surround area where the responses at a high frequency range are relatively enhanced under the steady background light because of increase in the mean light intensity. But such a contribution from the surround area is not sufficient to explain the enhancement observed in these experiments because of the following reason. As shown in Fig. 3, the amplitude of the response at low frequencies stays almost constant under different light intensities when the percentage of modulation is held constant. The steady annular background, on the other hand, reduces the percentage of light modulation in that area. The response from the surround where the background light at an intensity 10 times the control test light is applied will be about $\frac{1}{10}$ of the control. Under such circumstances, the response from the surround must be more than 20 times larger than that from the center in order to explain the augmentation of the response at high frequencies observed in these experiments. Such a ratio is quite improbable. Most of the effect of an

annular background which augments the response at a high frequency range, therefore, must be due to some other mechanisms.

In the other study of area effects, the area illuminated was simply reduced by passing the light beam through a hole in a black paper held near the surface of the retina. The gain characteristics of the S-potential to a light spot of about 1.0 mm diameter at a mean light intensity 1.0 log units above the standard are plotted by filled squares in Fig. 4. They show a marked attenua-
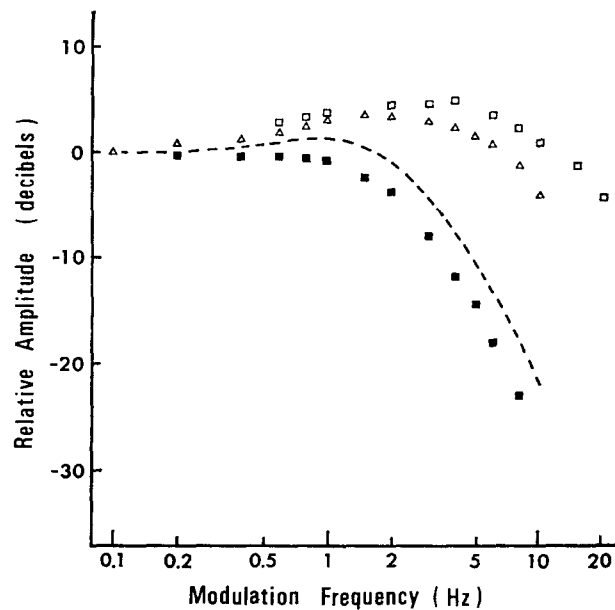

Figure 4 Effect of the area stimulated on the gain characteristics of the S-potential Open triangles: control data for diffuse illumination at an intensity 1.0 log unit above the standard. Open squares: responses to the same test light under the steady annular background illumination of 0.8 mm inner diameter at an intensity 10 times higher than the test light. The annular background augments the response at high frequencies. Filled squares: responses to a light spot of about 1.0 mm diameter at the same intensity as the control (1.0 log unit above the standard). The reduction of the stimulus area causes a marked reduction of the gain at high frequencies. The gain characteristics for the standard diffuse illumination, copied from Fig. 3, are also shown for comparison (broken line). The response amplitude at 0.1 Hz in each curve is set to 0 dB.

tion of the gain at high frequencies compared with the diffuse test light at the same intensity (open triangles). These two sets of data were obtained from different units in the same retina. Since all the S-potentials recorded in the same retina showed almost identical frequency characteristics under the same illumination condition, the difference seen in this figure is significant. The gain characteristic of the S-potential at the standard diffuse light is indicated by a broken line in Fig. 4 for comparison. It is shown in this figure that the

reduction of the area illuminated is similar in its effect to the reduction of the light intensity. In five horizontal cells studied for the area effect, the effect of reducing the stimulus area from diffuse to a spot of 1 mm was approximately equivalent to that of reducing the light intensity by a factor of 10. It is probable that some part of this effect is due to the scattering of light, since the spot is not strictly focused on the retina. But the area effect appeared too large to be ascribed to the effect of the scattered light alone. If the transmission of signals from receptors to horizontal cells is not modified by some interaction processes such as negative feedback or lateral interaction, the frequency characteristics of the S-potential should be dependent only on the intensity of light impinging upon receptors but not on the number of receptors illuminated. The area effect observed in these experiments suggests the contribution of some interaction process.

Although it is desirable to test all the area effect in the same horizontal cell, it was difficult with the present optical setup. However, the frequency characteristics can be estimated indirectly from the response to a step function. Generally, the transient response to a step function is closely related to the low frequency attenuation in the gain characteristics. In order to obtain further support on the area effect, therefore, the response of the horizontal cells to a step light was studied. When a step of diffuse light was superimposed on a steady diffuse background, the transient became more prominent with an increase in the background light intensity as was expected from the previous experiments. If the size of both test and background light was reduced, the transient became less prominent. The sample records of Fig. 5 illustrate this relation. These results are consistent with the previous observation on the frequency characteristics. It is also noted in Fig. 5 that the response to a light spot becomes considerably faster if the area of the background illumination is increased. The result indicates that the frequency characteristics of the horizontal cell response depend on the voltage level set by illumination.
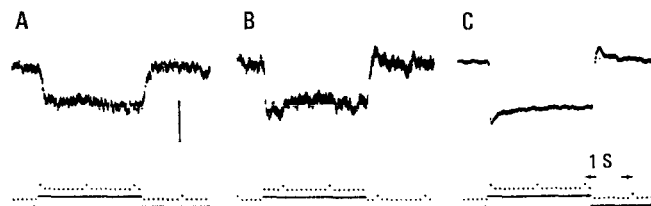

FIGURE 5   Responses of a horizontal cell to a square-pulse increment of the light intensity. (A) A response to a light spot of 1 mm diameter superimposed on a steady spot illumination. (B) A response to the light spot in the presence of a diffuse background illumination. (C) A response to a diffuse light in the presence of a diffuse background. All at the standard light intensity. Calibration is 1 mV in A and B, and 5 mV in C.

Bipolar cells

Bipolar cells, like horizontal cells, are second order neurons postsynaptic to receptors. Two types of bipolar cell responses have been reported in the teleost fish; the "on" center cells responding with depolarization to a small light spot at the electrode tip and with hyperpolarization to an annular illumination and the "off" center cells responding with hyperpolarization to a light spot and the depolarization to an annulus (Kaneko, 1970; Toyoda, 1973). Under dark-adaptation, all the on center cells showed a spectral response curve with a maximum at about 520 nm suggesting that they receive dominant inputs from rods, while most of the off center cells remained to show a maximum at about 620 nm and were considered to reflect cone activities (Toyoda, 1973). In order to rule out the contribution from the rod system, only the off center cells were subject to further analysis and the frequency characteristics of the on center cells were not studied in the present experiments. The off center cells, however, were rarely found in the dark-adapted retina. There were only three cells whose frequency characteristics were studied. One of them was stable enough to test the effect of selective adaptation.

The frequency characteristics of bipolar cells depend on the light intensity, as in the horizontal cells. The data shown with filled circles in Fig. 6 were obtained at the standard light intensity. There is a marked difference between the characteristics of the bipolar and the horizontal cells under diffuse illumination. The gain characteristic of the bipolar cell response shows a considerable attenuation at low frequencies and a peak near 5 Hz. A phase advance at low frequencies is also noted (filled circles). It is possible that the difference in the frequency characteristics of the horizontal and the bipolar cell is related to the difference in their receptive field organization. This possibility was tested from study of selective adaptation of the surround by means of a steady annular background of 0.8 mm inner and 4 mm outer diameter. The intensity of the background light was almost the same as the test light. The suppression of the surround response may not have been as complete as the selective stimulation of the center with a small light spot. However, the data obtained were conclusive enough to support the above possibility. The transfer characteristics of the bipolar cell response in the presence of the annular background do indeed look more like those of S-potentials. The data are plotted with open squares in Fig. 6.

From the gain and phase characteristics with and without selective adaptation, it is possible to obtain the transfer characteristics of the surround response (the component suppressed by the selective adaptation) by vector analysis. The data marked with open triangles in Fig. 6 were so obtained. The gain characteristics of the surround response of the bipolar cells was not markedly different from those of the center response except for a somewhat
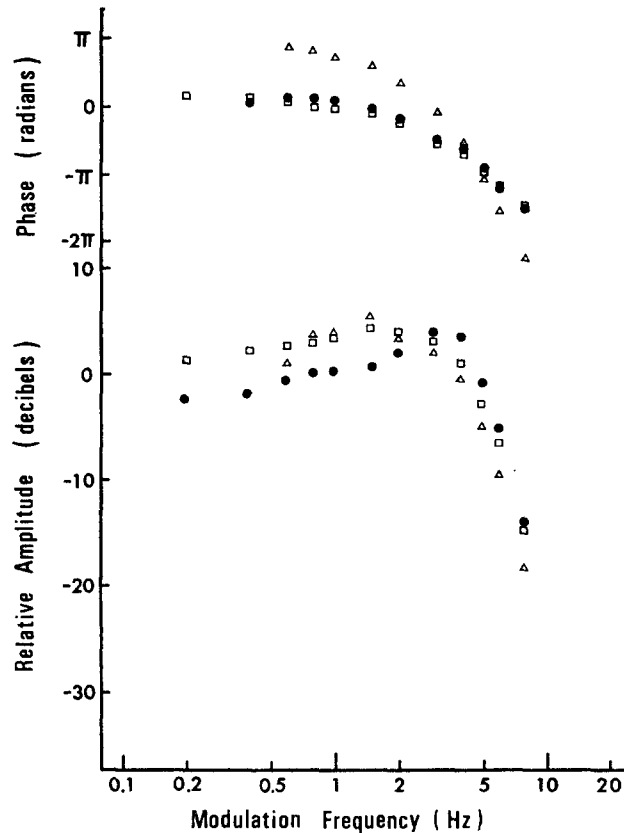
FIGURE 6   Gain and phase characteristics of a bipolar cell response. Filled circles: data for a diffuse illumination at the standard light intensity. Open squares: data obtained under a steady annular background of 0.8 mm inner diameter at an intensity almost equal to the test light. Open triangles: gain and phase characteristics of the surround component. Each point was obtained graphically from two vectors, one with and one without the steady annular background. It is seen that the surround response suppresses the center response at frequencies where their phase relationships are opposite, but augments it near 5 Hz where their phase relationships are the same.

greater attenuation at high frequencies. Their phase relations, however, were quite different. There is a phase difference of about 180° at low frequency. Then the surround response shows a phase lag increasing towards higher frequencies with a slope steeper than that of the center response. The result indicates more delay of the surround response compared with the center response. At about 5 Hz, the two responses show almost the same phase. Therefore, if both center and surround areas are illuminated, the surround response augments the center response at around this frequency and suppresses it at low frequencies where the two responses are about 180° out of phase.

Amacrine Cells

The amacrine cell responses were recorded in the light-adapted carp retina. They responded with either on or off or on-off responses to illumination. Some of them showed center-surround organization, and some did not (Toyoda et al., 1973).

The frequency characteristics of the three types of amacrine cells, on (open circles), off (filled circles), and on-off (filled triangles), are shown in Fig. 7. The total of nine amacrine cells, including two on and two off cells, were studied in the present experiments. In these cells, no center-surround organization was apparent.

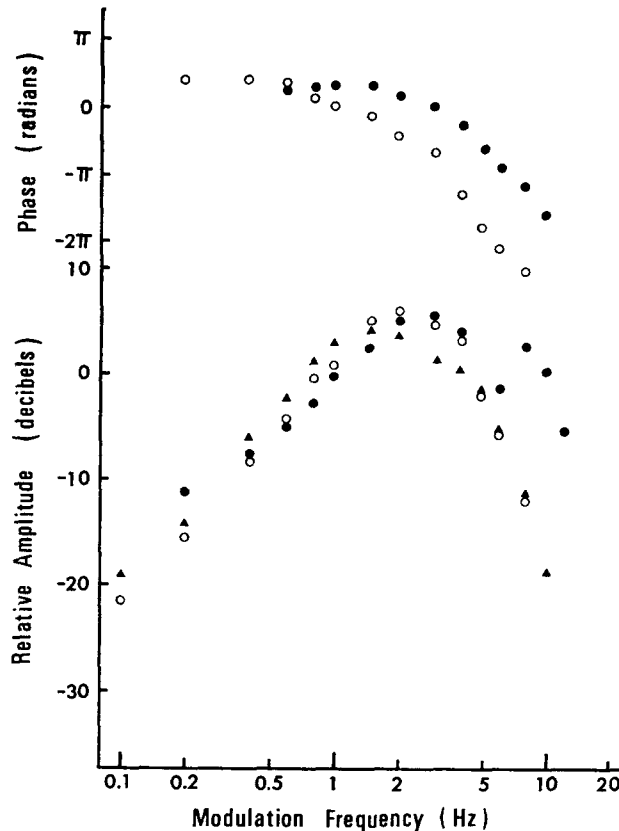However, it is possible that the annular light of 0.8 mm inner diameter

FIGURE 7 Gain and phase characteristics of amacrine cells. Filled circles: data from an "off" amacrine cell. Open circles: those from an "on" amacrine cell. Filled triangles: those from an "on-off" amacrine cell. Low frequency attenuation and phase advance at low frequencies are prominent in amacrine cells. The off amacrine cell shown in this figure exhibits two resonant peaks in its gain characteristics.

used in these experiments was too small to effectively elicit a surround response. The gain characteristics of the amacrine cells showed a marked low frequency attenuation even at the standard light level. The phase characteristics also exhibited a considerable phase advance at lower frequencies.

The response of the on-off cells was unique. The frequency of the sinusoidal response in these cells was twice the input frequency. The response often looked more triangular than sinusoidal. In a previous study on amacrine cell responses (Toyoda et al., 1973), it was concluded that the on-off response results from the combination of two opposing components: one depolarizing and one hyperpolarizing to illumination. In order to account for the frequency doubling, however, both depolarizing and hyperpolarizing components must be modified by a nonlinear process which is highly rectifying in its property. A rectifying process has also been proposed in the goldfish to account for the nonlinearity of the ganglion cell response (Spekreijse, 1969). In view of the contribution of a nonlinear process in the on-off amacrine cell, the applicability of the linear system analysis may be questioned. In one out of five on-off cells, the relation between the response amplitude and the modulation amplitude was studied with the result that the peak to peak amplitude was approximately linear to the modulation amplitude within a range used in the present experiments. The peak to peak amplitudes of an on-off cell were plotted in Fig. 7 (filled triangles) for comparison with the characteristics of other amacrine cell responses.

DISCUSSION

Factors Affecting the Frequency Characteristics of Receptors and Horizontal Cells
The visual cells of invertebrates such as the horseshoe crab exhibit a marked low frequency attenuation at high light intensities (see Dodge et al., 1968; Knight et al., 1970). This effect is considered to reflect an adaptation process of the receptors. The term adaptation process as used here refers to the *time-dependent* gain control mechanisms, which act as a high pass or a lead-lag filter. If the time constant of such an adaptation process is within a certain range, neither too fast nor too slow, it results in an apparent low frequency attenuation of the frequency characteristics. However, even at fairly high light intensities, the frequency characteristics of the distal PIII (mass receptor potential) and of a few intracellular records in the carp cones are usually flat over the low frequency range. Occasionally, a low frequency attenuation was observed in the distal PIII, but this is due to an incomplete isolation of the distal PIII from other ERG components which show a marked low frequency attenuation. In both receptors and horizontal cells, the response amplitude (or sensitivity) decreases immediately after a step increase of the background light intensity and recovers immediately after the return of the background light intensity to the previous level. This process of rapid gain control is

inherent in the mechanisms of transduction from the light intensity to the receptor potential which satisfies a nonlinear equation of the form V/V_{max} = $I/(I + K)$, where V is the response amplitude evoked by light of intensity I, K is the light intensity necessary to evoke the response of half the maximum amplitude V_{max} (see Naka and Rushton, 1966; Baylor and Fuortes, 1970). We do not know at present whether or not this process of transduction is in some way related to the time-dependent adaptation process. But, since the low frequency attenuation is not prominent in the carp cones, any gain control mechanism in cones must have a time constant quite different from that of the invertebrate photoreceptors.

Fuortes and Hodgkin (1964) simulated the photoreceptor response of the horseshoe crab by a series of low pass filters. They found that about 10 stages of filters are necessary to fit the initial phase of the response. Baylor et al. (1971) applied the same technique to the cone response of the turtle, and found that the response can be fitted by seven stages of filters.

The number of filters can be judged also from a slope of the gain characteristics curve in Bode plots at high frequencies. Spekreijse and Norton (1970), in their studies on the goldfish S-potentials, found that the gain decreases with a slope of 24 dB per octave at frequencies above 15 Hz. The gain characteristics of the carp S-potentials in the present experiments also show maximum slopes of 18–24 dB per octave at high frequencies, indicating that at least three or four stages of filters are necessary to simulate the S-potential. It may be worth noting, however, that these results, because of technical limitations, do not rule out the possibility that further stages of filters of faster time constant are involved. Although it is certain that low pass filters are involved in the transmission from receptors to horizontal cell, their contribution is not apparent in the present experiments. The difference in the gain characteristics of the receptor and the horizontal cell at the standard light intensity may not be significant, since no apparent difference is observed in the phase relation. The properties of an adaptation process may be simulated by an addition of one or more stages of a highpass filter with a transfer function k $(\tau_1 s + 1)$ or of a lead-lag filter with a transfer function k $(\tau_1 s + 1)/(\tau_2 s + 1)$. If the time constant τ_1 is nearly equal to or faster than the time constant of the low pass filters, the effect of the two will cancel each other, so that there will be no apparent low frequency attenuation in the gain characteristic curve. On the other hand, an addition of a high pass or lead-lag filter will attenuate the response amplitude at low frequencies but less at high frequencies, and results in a shift of the cutoff frequency towards higher frequencies.

The horizontal cell, on the other hand, shows a marked low frequency attenuation at high light intensities. It is prominent in the response to diffuse illumination, but becomes less prominent as the area illuminated is reduced.

If there were no interactions among receptors and horizontal cells, the frequency characteristics of the S-potential should be determined by those of receptors and should not be dependent on the area illuminated. It is unlikely that the low frequency attenuation comes from an adaptation process in the horizontal cell membrane, since the polarization of the membrane by current injected from the recording electrode did not affect the shape of the S-potential. On the other hand, the results can be explained by assuming a negative feedback mechanism from horizontal cells to receptors with a delay in the feedback loop. The delay may be due to low pass filters involved in the loop. The present results in the carp S-potentials differ from those of Spekreijse and Norton (1970) in the goldfish S-potentials in that no low frequency attenuation was observed in the latter. It is likely that Spekreijse and Norton used a light spot which was too small to affect the transmission from receptors through the feedback loop. The intensity range used in their experiments was about 0.6 log units and was perhaps not wide enough to prove the effect of light intensities.

In the turtle retina, Baylor et al. (1971) provided evidence for a negative feedback from horizontal cells to receptors, based on their intracellular observation that the hyperpolarization of a horizontal cell by current elicited depolarization of a receptor, and that the receptor of the turtle has an area effect; an increase in area illuminated results in a decrease in the amplitude of the receptor potential. To test the possibility of the negative feedback in the carp, the area effect of the receptor potential was examined in the present study with the result that some receptors though not so many as in the turtle showed the area effect. This area effect could possibly be ascribed to inhibitory synaptic inputs from horizontal cells to receptors, though we are in lack of morphological as well as direct physiological evidence.

A negative feedback from horizontal cells to receptors has been suggested also in the catfish based on a result of nonlinear analysis of the S-potentials (Marmarelis and Naka, 1973). The negative feedback plays a role in broadening the frequency characteristics of receptors. Unfortunately, however, we were unable to study the frequency characteristics of the carp receptors having an area effect because of unstable intracellular recordings. If the low-frequency attenuation observed in horizontal cells is mainly due to the negative feedback from horizontal cells to receptors, the response recorded intracellularly from receptors having an area effect should also show a low frequency attenuation. From an analysis of the extracellular potential field produced by photocurrent and by synaptic current from proximal portion of receptors, it is concluded that the absence of the low frequency attenuation in the distal PIII does not necessarily exclude the presence of a low frequency attenuation in some receptors.

Factors Affecting the Frequency Characteristics of Bipolar and Amacrine Cells

The frequency characteristics of the bipolar cell response to diffuse light differ from those of the S-potential in that the attenuation of the gain at low frequencies and the corresponding phase advance are more marked than in the S-potential. It was shown in Results that this difference comes mainly from their different receptive field organization. The bipolar cell response consists of two antagonistic components: one from the center and one from the surround. Illumination of the area where the dendrites of a given bipolar cell make synaptic connections with receptor terminals elicits the center response (Werblin and Dowling, 1969; Kaneko, A., personal communication). Since the dendrites of both bipolar and horizontal cells make synaptic contacts within the same receptor terminal forming a triad structure (Stell, 1967; Witkovsky and Dowling, 1969), it may be reasonable to assume that the mechanism of synaptic transmission from receptors to these two kinds of second order neurons are the same. However, the frequency characteristics of the center response of the bipolar cell observed in the present experiments are not identical with those of the S-potential but somewhat broader. It is likely that the difference is due to contamination of the center response by the surround component not completely suppressed under the steady annular background.

The surround response is slower than the center response, judging from the surround's steeper high frequency cutoff and more prominent phase lag at higher frequencies. Even if both response were to have the same gain characteristic curve, a difference in their latencies must play an important role in determining the overall transfer characteristics of the bipolar cell response to diffuse light. Suppression of the gain at low frequencies and an augmentation near 5 Hz can be ascribed to the latency difference (see Ratliff et al., 1967; Ratliff et al., 1969). If we assume that the difference in the phase characteristics of the center and the surround response is due solely to the difference in their latencies (dead time), the phase lag of π radians at 5 Hz corresponds to the latency difference of 100 ms. However, the actual time difference of the rising phase of the response was 30–40 ms, although it was somewhat greater when measured at the peak of the response. These observations indicate that the latency difference is not sufficient to explain the phase difference. The delay in the response is possible if the response is modified by further stages of low pass filters, which also make the high frequency cutoff of the gain steeper. Thus the difference in the phase characteristics observed in the present experiments appears to be due to the slow time course of the surround response and also partly to the latency difference. It may be reasonable to assume further stages of filters for the surround response, because it appears to be mediated

by an interneuron, the horizontal cell (see Werblin and Dowling, 1969; Toyoda, 1973).

The gain characteristics of the amacrine cell responses show more marked low frequency attenuation than those of the bipolar cell response. There is also a marked phase lead at low frequencies. Moreover, not only the on-off response, in which the nonlinear property is necessary to account for its sinusoidal response of twice the input frequency, but also other amacrine cell responses contained more harmonic components than the bipolar cell responses especially at a low frequency range. It is considered that the low frequency attenuation, the phase lead and the nonlinearity are all related to a process designated here as the neural adaptation. It is difficult to acribe all these phenomena to interactions or to feedback mechanisms, although it is also difficult to rule out the contribution of these two mechanisms. The amacrine cell responses shown in Results did not show a center-surround antagonistic organization. However, even if they do not show such an organization, most amacrine cell responses have an opponent component within the same small area in the receptive field (Toyoda et al., 1973). Thus the interaction of the two opposing components may play some role in the frequency characteristics of the amacrine cell responses. In their study of the transfer function of retinal ganglion cells, Maffei et al. (1970) predicted a second resonant peak in the gain characteristic curve by a combination of the center field and the antagonistic surround field. The double peak observed in the off amacrine cell as illustrated in Fig. 7 looks close to their results, and could be due to the interaction of the two opposing components.

The ganglion cells and other neurons in the central visual pathways were not included in the present experiments. However, from the comparison of the transfer functions of the ganglion cells studied in the fish retina by Schellart and Spekreijse (1972) and of the bipolar and amacrine cells studied in the present experiments, it is concluded that the main features of the dynamic characteristics in the ganglion cell are already seen in the bipolar and especially in the amacrine cell. The neural adaptation and interaction, playing an important role of modifying the frequency characteristics in bipolar and amacrine cells, however, will modify the visual information further in ganglion cells and in other higher order neurons.

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