Dynamic Characteristics of Retinal Ganglion Cell Responses in Goldfish

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ABSTRACT   A cross-correlation technique has been applied to quantify the de-
pendence of the dynamic characteristics of retinal ganglion cell responses in
goldfish on intensity, wavelength, spatial configuration, and spot size. Both
theoretical and experimental evidence justify the use of the cross-correlation
procedure which allows the completion of rather extensive measurements in a
relatively short time. The findings indicate the following. (a) The shape of the
amplitude characteristics depends on the energy per unit of time (power) falling
within the center of a receptive field rather than on the intensity of the stimulus
spot. For spot diameters of up to 1 mm, identical amplitude characteristics can
be obtained by interchanging area and intensity. Therefore the receptor
processes lo not contribute to the change in the amplitude characteristics as a
function of the power of the stimulus light. (b) For high frequencies the ampli-
tude characteristics obtained as a function of power join together in a common
envelope if plotted on an absolute sensitivity scale. For spontaneous ganglion
cells this envelope holds over a range of three log units and the shape is identical
for central and peripheral processes. (c) The amplitude characteristics of the
central and peripheral processes converging to a ganglion cell are identical,
irrespective of the sign (on or off) and the spectral coding of the response.
Therefore we have no evidence for interneurons in the goldfish retina unique to
the periphery of the receptive field.

INTRODUCTION

Analyses of the wavelength dependence of ganglion cell spike discharges and
sustained graded responses of horizontal cells showed that in goldfish and
carp retina the spectral codings of both types of responses were identical
(41, 47).[1] However, the spatial coding of ganglion cells proved to be more
complicated. Unlike the horizontal cells, the ganglion cells show an antag-
onistic center-periphery organization (4, 32). Furthermore, the amplitude
characteristics of the three types of horizontal cell responses (monophasic,

[1] Spekreijse, H., and A. L. Norton. 1972. The color-coding of S-potentials. Manuscript in prepara-
tion.

biphasic, and triphasic) are alike irrespective of the wavelength of the stimulus light (38).

In this paper we will pay attention to the dynamic properties of the ganglion cell response. In particular there will be a determination of the dependence of the dynamic characteristics on wavelength and spatial configuration of the stimulus light. In order to demonstrate those relationships it was necessary to use a cross-correlation procedure, so as to gather the rather extensive measurements within the short lifetime of the ganglion cells in an isolated retina. The nonlinear responses of ganglion cells required extensive cross-validation in the application of this method. The theoretical justification of the cross-correlation technique is given in Methods, and experimental tests of its applicability are presented in Results, sections 1 and 2.

The present study was undertaken to work out in greater detail the dynamic characteristics of ganglion cells. This detail might be useful in evaluating a number of recent reports on similarities between human psychophysical and infrahuman electrophysiological observations. There have been numerous quantitative studies of the relation between stimulus parameters and dynamic characteristics of single cell responses. The original studies were performed on cat retina (3, 13, 16) and in the invertebrate eye (10, 22, 23, 34). A recent example, which is particularly interesting for the present study, is the observation by Maffei et al. (28) that the amplitude characteristics of the response of ganglion cells in cat retina depend on spatial configuration. He found that, irrespective of the "sign" ("on" or "off") of the response, the cut-off frequency of the peripheral process lay at a lower value than the cut-off frequency of the central process. Maffei's findings are consistent with human psychophysical data on temporal and spatial contrast sensitivity (25, 36).

In a very different preparation, the lateral eye of *Limulus*, the dependence of the dynamic characteristics on another parameter, stimulus intensity, has been studied. In *Limulus* both the generator potential (14, 33) and the quantal bumps (11) showed different dynamic properties depending on the average intensity used. An increase in mean intensity resulted in a shift of the cut-off frequency to higher values and in a sharpening of the amplitude characteristics. Similar results were obtained in a recent study on the amplitude characteristics of the discharge pattern of geniculate body cells in monkey (40). The dynamic properties of these monkey cells show a close correspondence with human psychophysical data on flicker perception.

Starting with human psychophysics, we find a number of studies that have important electrophysiological implications. The pioneering studies by De Lange (6, 7) have established a relation between flicker fusion in man and photopic retinal illumination. These studies have demonstrated that the shapes of the human psychophysical flicker fusion curves are a function of the

mean intensity of the flicker light. Moreover, Van der Tweel (44) and Kelly (18) have shown that such De Lange curves are a function of the spot size used. The importance of spot size has also been demonstrated for discharge patterns in the *Limulus* eye (21, 35). All of these observations suggest that spatial interaction plays an important role in the determination of the shapes of the dynamic characteristics.

In this paper experiments will be presented to quantify the dependence of the dynamic characteristics of ganglion cell responses not only on intensity, stimulus configuration, and spot size,[2] but also on stimulus wavelength. The latter relation is necessary because psychophysical observations have resulted in models with wavelength-dependent dynamic properties (8, 15, 20, 46). To evaluate these wavelength-dependent models the goldfish retina is an almost ideal preparation, since it has been shown (39, 41, 45) that the various components of the goldfish ganglion cell response are related to the wavelength of the stimulus. The action spectrum of each wavelength-coded component is similar (41) to the absorption spectrum (27, 30) of one of the three types of cones. This indicates an original separation of each component, although various combinations of them occur at the ganglion cell level.

METHODS

Common goldfish (*Carassius auratus*), 6–8 inches long, were used in all experiments. Dark-adapted retinas were dissected from enucleated eyes and placed receptor side up in a temperature-controlled chamber. The temperature of the chamber was kept at 14°–19°C throughout the experiments. During a particular experiment the temperature of the isolated retina was maintained constant (within 0.2°C) because its dynamic characteristics were found to depend strongly on temperature. A moist gas mixture (95 % oxygen, 5 % carbon dioxide) was passed over the preparation. Light for stimulation came from below and the microelectrode entered the retina from above.

Two electronically controlled television projection tubes (Philips MW 6/2) served as sources for two independent light stimuli. The phosphors had broad emission spectra and gave a color temperature of about 6500°K. The intensities of the two light sources could be modulated independently (9). Photocells monitored and controlled the outputs of the projection tubes. The two optical pathways were combined by a beam splitter. Stimulus intensity was limited to a maximum of about three log units above the ganglion cell absolute threshold.[3] The pathway of each stimulus had a collimated region where interference filters were inserted to select the stimulus wavelength. Intensity was set by neutral density filters. An aperture stop in each pathway,

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[2] It has been shown (41) that in the goldfish retina the diameter of the receptive field center is constant irrespective of the spectral coding of the central processes and irrespective of the presence of peripheral processes. This diameter, as determined by the area of full summation, extends up to 1 mm in the plane of the retina. Therefore it is sufficient to quantify the dependence of the dynamic characteristics as a function of absolute, and not of relative, spot size.

[3] Comparisons between the absolute thresholds of ganglion cell responses in goldfish as a function of spectral and spatial coding can be found in Spekreijse et al. (41).

which could be varied in size, shape, and position, was focused upon the retina. In this way spots, anuli, and slits could be imaged at the plane of the receptor layer.

Recordings of ganglion cell spike potentials were made with platinized platinum-iridium microelectrodes (48). The signals from the microelectrode, which was centered with respect to the stimulus field under microscopic control, were detected through conventional electronic amplification. The output of the amplifier and the signals of the photocells were displayed on an oscilloscope, and both were also fed to a tape recorder.

The shaped spike responses to all periodic stimuli were averaged on-line by means of a special purpose computer (Biomac 1000, Data Lab. Ltd., London). The number of summations was 50–1000, depending upon the strength of the ganglion cell discharge. A final record was obtained by plotting the output of the computer with an X-Y recorder.

The average response computer was also utilized to obtain the cross-correlation function between the randomly modulated light stimulus and the shaped spike responses. In the on-line calculation the synchronization pulses, which initiated the computing cycle, were directly derived from the noise stimulus. The trigger pulses were generated whenever the Gaussian noise exceeded a preset threshold and the shaped spikes were summated with a sweep time of 320 msec (*triggered correlation*). In this condition the computer output gives an over-all impression of the true correlation function (5). We found this on-line procedure useful since (*a*) the effect of a change in the stimulus condition upon the dynamic characteristics could be observed directly and (*b*) an estimate of the quality of the correlation function could be obtained, permitting us to keep the duration of a given condition in the experimental sequence as short as possible.

Cross-Correlation

The cross-correlation function is a useful tool to study input-output relations of a system. The finite-time estimate $\Phi_{xy}^*(\tau)$ of the cross-correlation function $\Phi_{xy}(\tau)$ is defined as:

$$\phi_{xy}^*(\tau) = \frac{1}{T} \int_0^T x(t)y(t + \tau) \, dt,$$

where τ is the independent time-delay variable, T is the integration time, and $x(t)$ and $y(t)$ are the two signals studied.

With white noise (zero mean and unity variance) as the input signal $x(t)$ to a linear system with a unit impulse response $h(t)$, and $y(t)$ as the response, it can be shown (24) that the input-output cross-correlogram represents the impulse response: $\Phi_{xy}(\tau) = h(\tau)$. Fourier transformation of $h(\tau)$ gives directly the amplitude and phase characteristics of the linear system. Definitions of these characteristics can be found in basic textbooks on system analysis and also in, for example, Spekreijse and Norton (38), where the dynamic properties of horizontal cell responses have been determined.

Some caution is required in applying this method to the analysis of the spike discharges of retinal ganglion cells to white noise–modulated light.[4] The descriptive

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[4] In reality the frequency band of the noise stimulus is only flat over the frequency range which is of importance for the system analyzed. For the analysis of the discharge patterns of the goldfish

classification of ganglion cell responses into "on," "off," and "on-off" types indicates that the discharge patterns reflect strongly nonlinear processes. It has been shown (37) that to a first approximation the nonlinearities can be ascribed to rectifying processes with a static nature. This means that the distortion in the response of the ganglion cells depends only on the amplitude and not on the frequency content of the stimulus. It has been shown that even for a system containing a static, single-valued nonlinearity, the over-all impulse function $h(\tau)$ of the linear elements in such a system can still be measured with the cross-correlation method (2). With $x(t)$ a white noise (zero mean and unity variance), one finds $\Phi_{xy}(\tau) = Ch(\tau)$ where C is a constant determined by the characteristic of the nonlinearity. Therefore, if the assumption about the static nature of the retinal nonlinearities holds, the correlation method might be applied to determine the dynamic properties of the retinal transformations. It should be noted, however, that the retinal transformations upon the stimulus light finally result in a series of spikes. With regard to the static nature of the nonlinearities, this implies that the probability of occurrence of a spike is assumed to have a strict relation to the instantaneous value of the stimulating signal at the site relevant for the spike generation. This assumption holds to a first approximation for the ganglion cell discharges in the goldfish retina (37).

Calculation of the cross-correlation function implies multiplication of two signal values. In our situation this operation is greatly simplified since the noise stimulus $x(t)$ has to be multiplied with either zero (no spike) or one (spike with unit area). This operation can easily be performed with a special purpose computer. The nerve impulses are used as trigger pulses and the corresponding waveform sections of the stimulus noise are averaged. Since the spikes are elicited by the noise stimulus, noise stimulus sections before the spike response must be averaged. This can easily be done by replaying the taped records in reverse (*reverse triggered correlation* [5]). This operation can also be performed on-line by a general purpose computer. The relevant stimulus waveform sections are stored up till the moment a nerve impulse has been generated. Corresponding waveform sections are then averaged to obtain the cross-correlogram.

The cross-correlograms presented in this paper were determined on a PDP-9 computer. The cross-correlograms had a length of 1 sec and consisted of 256 points. The computer has also been used to obtain the Fourier transform of $\Phi_{xy}^{*}(\tau)$ which shows the amplitude and phase characteristics. No smoothing procedures have been applied. Particularly with the cross-correlation procedure, the reliability of the characteristics at the low frequency end is strongly reduced. This is also the case with the sine wave method, but there it is customary to increase the stimulus duration with decreasing frequency.

RESULTS

Before analyzing the dynamic characteristics of the spike discharges in the goldfish retina, we first classified the ganglion cell responses according to their spectral and spatial coding by monochromatic, squarewave stimulation with a frequency of 0.5 Hz and 100% modulation depth. It proved useful for the

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ganglion cells a frequency band of 0-50 Hz proved to be sufficient within the temperature range used.
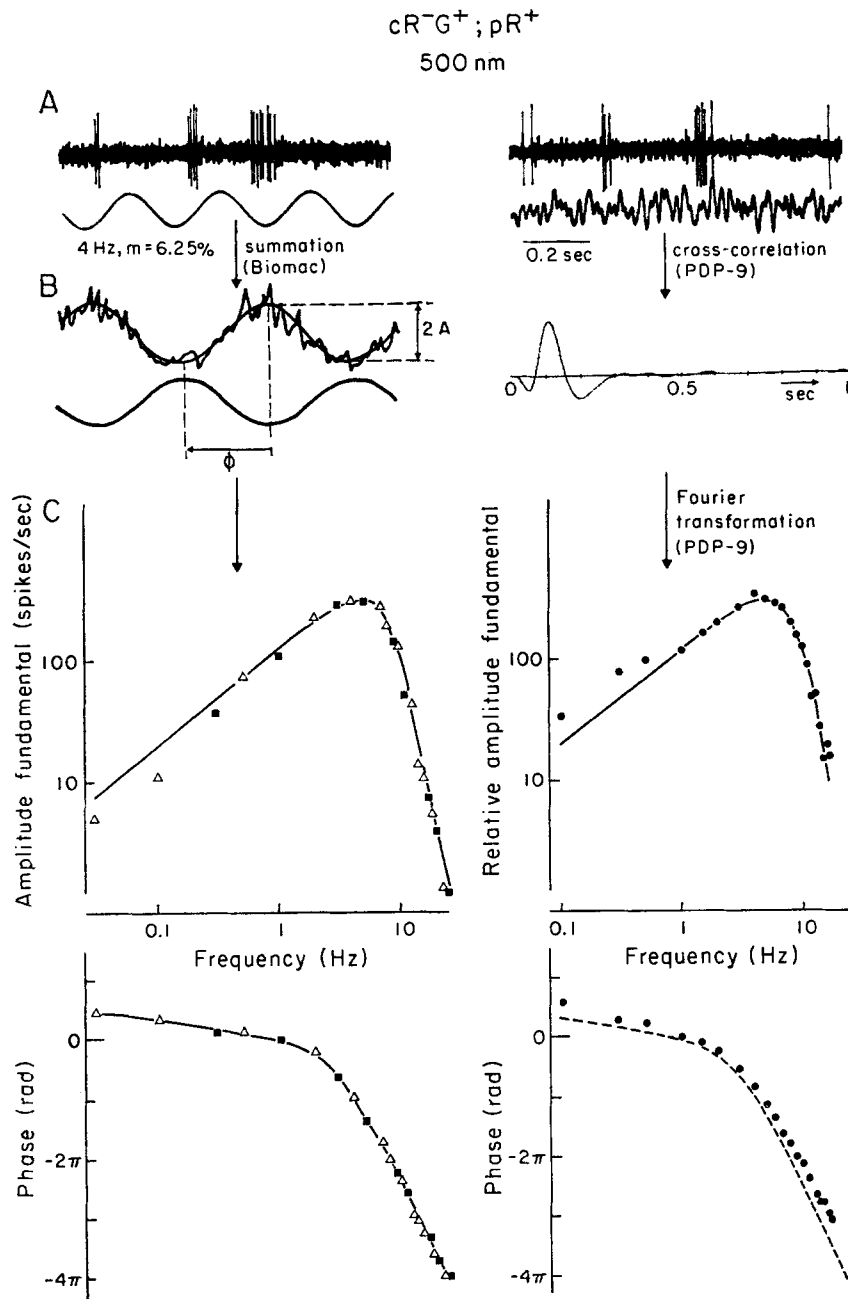
FIGURE 1.   Data analysis of ganglion cell responses to sinusoidally (left column) and Gaussian noise (right column)–modulated light. Row A gives an example of the response to either of the two stimuli. Summation of 300 periods of the shaped spike discharges to sine wave–modulated light (4 Hz) results in the averaged response depicted in row B. Measurement of the fundamental component in this response gives one point of the amplitude characteristic. Likewise the phase difference between stimulus and averaged

evaluation of the experimental results also to take into account the spontaneous firing level of the ganglion cells. More or less arbitrarily we distinguished (*a*) silent cells wih firing rates in the dark of less than approximately 1 spike/sec and (*b*) spontaneous cells which fire under the same conditions at rates of up to 30 spikes/sec. Both types of cells can be found in the same retina and sometimes they can even be detected simultaneously with the same electrode. The average spike responses of the silent cells to sinewave light had more or less a half–wave-rectified character (37), whereas the spike density distribution of the spontaneous cells followed to a large extent the shape of the input sinusoid.

It is also essential to classify ganglion cells according to their dynamic behavior upon the onset or the cessation of the stimulus light. Most of the discharge patterns found in the goldfish retina are phasic. Phasic cells respond with a short burst of spikes to a step in intensity. They perform a differential transformation upon the stimulus, the time constant being of the order of 25–50 msec (37). Occasionally ganglion cell responses can also be found with much longer time constants, up to 100 sec. To casual observation such responses may appear to be tonic. However, true tonic cells are very seldom observed in the goldfish retina. Since ganglion cells with long time constants were so rarely found, we will neglect them in this paper.

1. *Dynamic Characteristics Determined with Noise and Sine Wave Stimuli*

In Methods we described the correlation procedure to determine the unit impulse response of retinal ganglion cells. Fourier transformation of this impulse response gives the amplitude and phase characteristics. On the other hand, amplitude and phase characteristics can also be determined directly by stimulation with light modulated sinusoidally at various frequencies.

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response gives one point of the phase characteristic. Repeating the measurements for various frequencies results in the amplitude and phase characteristics depicted in row C (left column). Initially a sequence of increasing stimulus frequencies was used. The corresponding data points are indicated by (■). Next a decreasing sequence of frequencies was applied, which resulted in the (△) data points. The similarity between both sets of data indicates that during this 1.5 hr experiment the ganglion cell remained in a stationary condition. The sine wave stimulus sequence was four times interrupted by 2-min noise stimulations with a variance (stimulus strength) of 40%. An impression about the efficiency of the correlation method can be obtained by comparing the total duration of the experiment with the 8 min noise stimulation. The cross-correlogram obtained from this 8 min response sample is depicted in row B. Fourier transformation resulted in the amplitude and phase characteristics depicted in C (right column). The values along the vertical axis of the amplitude characteristic are adjusted to those found with sine wave–modulated light. The diameter of the central stimulus spot is 1 mm at the plane of the retina. The temperature of the preparation is 16.1°C. The mean stimulus intensity after passing through an Ealing TFP 500 nm interference filter amounts to 20 nw/cm². In all experiments Ealing interference filters are used, except where otherwise noted.

Fig. 1 illustrates the signal control and data analysis that are required for each of the two stimulus forms in order to obtain those characteristics. The left column shows the spike response of a spontaneous phasic ganglion cell to a green light modulated with a 4 Hz sine wave. Summation of the shaped spike responses over a large number of periods of the sinusoidal stimulus results in the spike density distribution depicted in B. The sinusoidal shape of this spike distribution indicates that the probability of occurrence of a spike depends only on the instantaneous value of the stimulating signal at the site relevant for its generation.[5] Measurement of the amplitude ratio of the input sine wave (modulation and depth) and the fundamental component in the average spike density distribution gives one point of the amplitude characteristic. Likewise the phase difference between these two signals gives one point of the phase characteristic.

In the right column of Fig. 1 the various transformations upon the spike response of the same ganglion cell to a Gaussian noise stimulus are presented. The mean intensity of noise and sine wave stimuli were the same. With the triggered correlation method the unit impulse response of the ganglion cell can be obtained directly. Fourier transformation of this impulse response gives the amplitude[6] and phase characteristics that are depicted in the bottom half of the right column.

The two sets of amplitude and phase characteristics look very similar. However, the dynamic characteristics determined with the noise stimulus scatter at the low frequency end, due to the rather short stimulus durations chosen. Moreover, the phase characteristic shows a slight but consistent phase lead. As indicated in Methods, the identity of amplitude and phase characteristics measured with the triggered correlation procedure with those determined directly with sine wave modulated light depends upon the validity of the assumption that the retinal nonlinearities are of a single-valued, zero-memory type. The similarity between the two sets of data indicates that this assumption is justified.

2. Dynamic Characteristics as a Function of Depth of Modulation

Another point of importance is the effect of stimulus strength on the shape of amplitude and phase characteristics. In Fig. 2 the amplitude of the fundamental component is depicted as a function of modulation depth for various frequencies of the sinusoidally modulated stimulus. The right column gives the data for a spontaneous ganglion cell and the left column shows a similar

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[5] Occasionally ganglion cells also have been observed with spike discharge patterns that are phase-locked to the input signal. The analysis of these multimodal density distributions remains a point for further investigation.

[6] The scaling along the vertical axis of this amplitude characteristic is not in absolute units, since the weighing effect of the nonlinearities upon the cross-correlogram is unknown (see Methods).

set of data for a silent ganglion cell. As is evident from the data in the upper half of the figure, a linear relation holds between the response strength and the amplitude of the input sine wave. However, for relatively high modulation
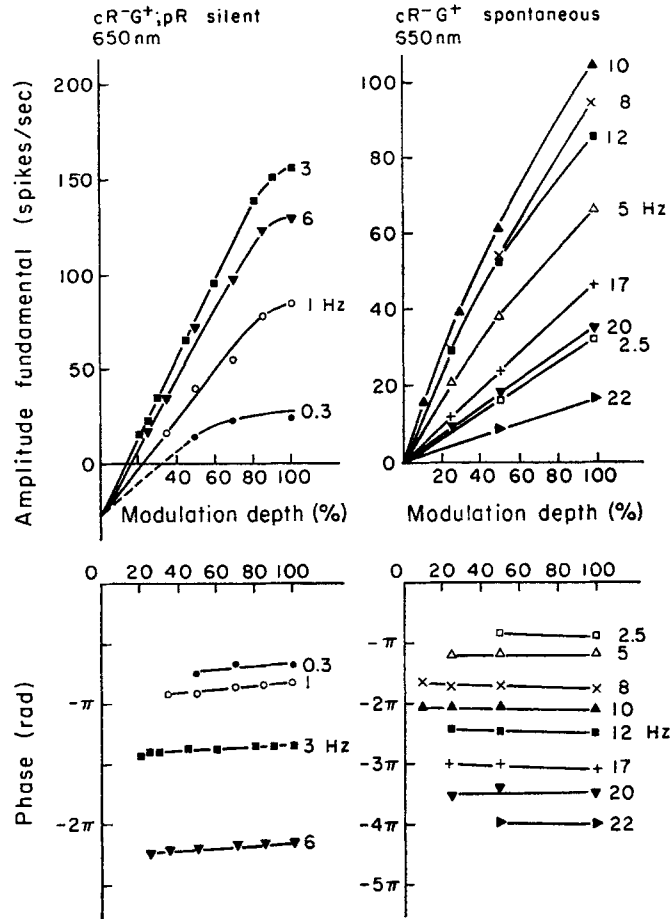

FIGURE 2.   Both for a silent and for a spontaneous ganglion cell the amplitudes of the fundamental component in the averaged responses are plotted as a function of modulation depth for various frequencies of the sinusoidal stimulus (note that the silent ganglion cell exhibits a threshold). Likewise the phase shifts are plotted in the bottom half. They are quite independent of modulation depth. The stimulus intensity is 60 nw/cm². The spot diameter is 0.6 mm for the silent and 2 mm for the spontaneous cell. The temperatures are 15.0°C and 19.0°C, respectively.

depths a deviation from linearity can generally be observed. For the spontaneous ganglion cells this deviation is due to clipping (zero spike frequency), while for the silent ganglion cells the distortion can be attributed to saturation. At low modulation depth the silent ganglion cells exhibit a threshold. The extrapolated straight lines for modulation depths below this threshold

all cross the vertical axis at the same point. This suggests that the threshold is frequency independent and that the nonlinearity is approximately static near threshold. On the other hand the slopes of the curves are frequency
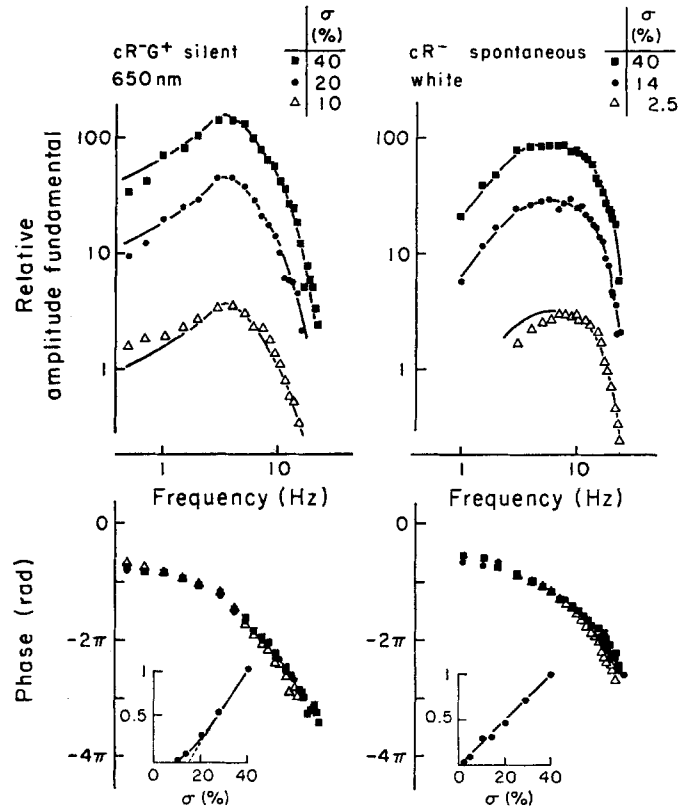

FIGURE 3.   Amplitude and phase characteristics of a silent (left column) and a spontaneous (right column) ganglion cell as a function of the strength (variance) of the Gaussian noise–modulated light. The shape of the characteristics is quite independent of stimulus strength. The inserts in the lower graphs show the shift of the amplitude characteristics along the vertical axis necessary to get the curves superimposed. Also, here the silent cell exhibits a threshold. For clarity only the characteristics obtained at the highest, the lowest, and an intermediate stimulus strength are shown. For the silent cell the mean intensity of the red stimulus light amounts to 60 nw/cm², the diameter of the central stimulus spot is 1.2 mm, the temperature is 16.5°C, and the signal samples are approximately 9 min. For the spontaneous cell these values are 100 nw/cm², 1 mm, 17.3°C, and 5 min, respectively, and the stimulus has a broad spectrum.

dependent. They are mainly determined by the dynamic properties of the retinal processes preceding the ganglion cells (37).

The lower part of Fig. 2 gives the phase shift for various frequencies as a function of modulation depth. As can be seen this phase shift is quite inde-

pendent of modulation depth and depends primarily on stimulus frequency. These data also demonstrate that the amplitude and phase characteristics determined with sine wave stimuli are quite independent of the strength of the sine wave stimulus.

Fig. 3 compares the amplitude and phase characteristics as determined for both a spontaneous and a silent ganglion cell with Gaussian noise–modulated light. The amplitude and phase characteristics of both sets of data are fairly independent of stimulus strength. For low stimulus frequencies, all of the phase characteristics tend to reach a phase lag of $-\frac{1}{2}\pi$, i.e. $(-\pi + \frac{1}{2}\pi)$. The off sign of the response accounts for the first term $(-\pi)$. The $+\frac{1}{2}\pi$ is the value extrapolated in accordance with the differentiative transformation which is characteristic for phasic ganglion cells.

The inserts in the lower graphs of Fig. 3 show the shifts along the vertical axis of the amplitude characteristic that are required to have the curves overlap. For the spontaneous ganglion cell this relation is linearly proportional to the stimulus strength, which is consistent with the sine wave data depicted in Fig. 2. For the silent ganglion cell the corrective relation is a more complicated function, in accordance with the threshold of silent units.

The observations, presented in the previous two sections, indicate that the assumption about the simple static nature of the nonlinearity holds. The similarity between the sine wave and noise data and the independence of the characteristics on stimulus strength justify the use of the cross-correlation method. We prefer the noise stimulus to the sine wave stimulus because the latter only allows testing with sequential frequencies, whereas with the noise stimulus all frequencies are applied at the same time. Therefore noise stimulation not only reduces the effect of an over-all change in sensitivity of the ganglion cell on the shape of the amplitude characteristic but also allows for the completion of the measurements in a rather short time.

3. *Dynamic Characteristics as a Function of Intensity*

The cross-correlation method was used to derive the amplitude and phase characteristics as a function of the mean intensity of the noise stimulus. The top half of Fig. 4 gives the amplitude characteristics for a silent and a spontaneous ganglion cell. As is evident from this figure with constant modulation depth (variance) the response strength increases with intensity. Particularly for frequencies above 10 Hz the response seems to grow proportionally with intensity. The major difference between the two sets of data is the shift along the vertical axis as a function of mean intensity. For low intensities this shift was larger for silent than for spontaneous ganglion cells. A reason might be the threshold of silent ganglion cells. Such a threshold expresses itself in the weighting constant C which multiplies the "real" unit impulse response (see Methods). The stimulus has to exceed the threshold before a spike discharge

can be elicited. At lower stimulus intensities the probability of exceeding the threshold is less than at higher intensities. This effect of the threshold will be pronounced in the figures because the percentage of fluctuation around the mean stimulus intensity, rather than the absolute amplitude of the stimulus,
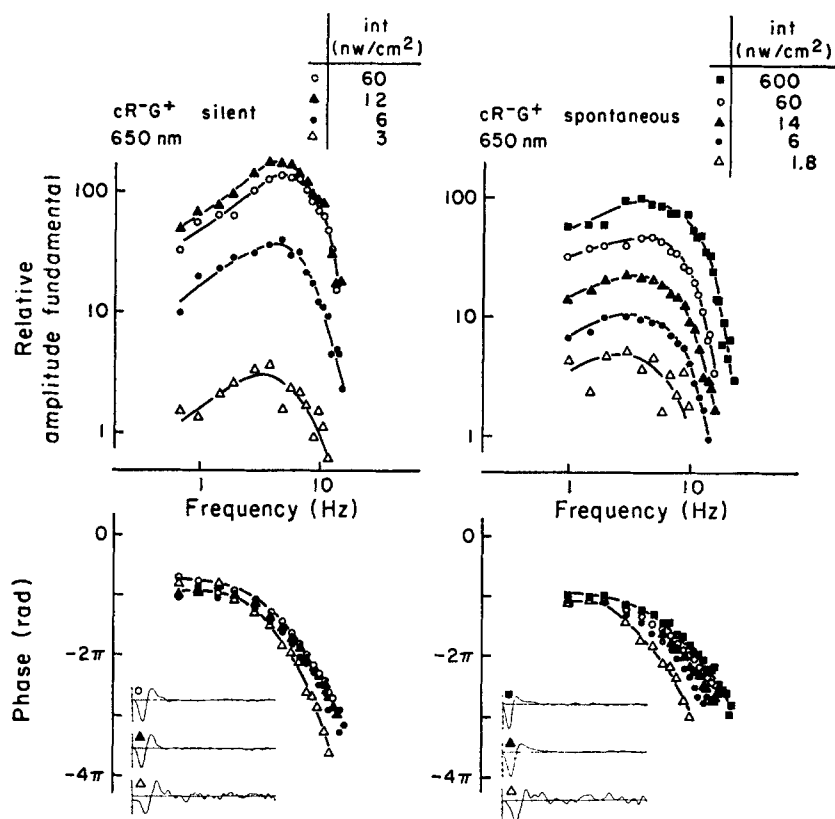

FIGURE 4. Amplitude and phase characteristics of a silent and a spontaneous ganglion cell are shown as a function of the mean intensity of the Gaussian noise–modulated (σ = 40%) light. In the inserts in the bottom graphs the normalized cross-correlograms (1 sec duration), obtained from signal samples of about 5 min, are depicted for three of the intensities used. For the silent unit the spot size is 1 mm and the temperature is 16.0°C. For the spontaneous unit these values are 1.2 mm and 17.1°C, respectively.

was used as a parameter. Furthermore, for both types of ganglion cells there is an upper limit above which an increase in intensity no longer results in an increase in response amplitude. This phenomenon initially appears at frequencies below 10 Hz. At still higher intensities the response of the silent ganglion cell even exhibits a reduction in amplitude.

The phase characteristics, which correspond to the amplitude characteristics shown in the top half, are plotted in the bottom half of Fig. 4. With increasing intensity the steepness of the phase characteristics becomes less

pronounced. This suggests a reduction in latency with increasing intensity, since phase shift and latency are related to each other with frequency as the proportionality factor: $\phi = -\omega\tau$. The change in latency with intensity cannot be directly determined from the phase characteristics, however, because the corresponding amplitude characteristics also change with intensity. Since the amplitude and phase characteristics are directly related to each other, a change in the amplitude characteristic implies a change in the shape of the phase characteristic (24). On the other hand, an estimate of latency can be obtained from the correlogram, although this measurement is sometimes difficult. This is illustrated in the small traces inserted at the bottom of Fig. 4 where the cross-correlation functions are presented for the highest, the lowest and an intermediate intensity used.

The change in the shapes of the amplitude and phase characteristics with intensity was also observed with sine wave–modulated light. These changes proved to be independent of the sign (on or off) of the response, the wavelength of the stimulus light, and the stimulus configuration (spot or anulus). Moreover, the shapes themselves are independent of the sign of the response, in accordance with the findings of Maffei et al. (28) in the cat retina.

4. Dynamic Characteristics as a Function of Stimulus Wavelength

The amplitude and phase characteristics of the two spectrally coded components in a silent phasic ganglion cell with red-off, green-on central processes are depicted in Fig. 5. These characteristics were determined for various intensities of a sinusoidally modulated monochromatic light. As can be seen, the changes in the shapes of the amplitude characteristics with intensity exhibit the same features as described above for noise-modulated light (Fig. 4). A comparison of the amplitude characteristics of the red and the green components shows that the shapes of the amplitude characteristics are independent of the spectral coding of the particular component. However, the intensity of the stimulus light is not the only parameter which must be compared. We obtained similar amplitude characteristics for different mean intensities. This might be caused by the difference in relative sensitivity between red and green components, since equally strong responses have identical dynamic properties.

The bottom half of Fig. 5 gives the corresponding phase characteristics. Extrapolation of these characteristics to lower frequencies suggests a phase shift of $-\frac{1}{2}\pi$ for the red and $+\frac{1}{2}\pi$ for the green component. Again, taking into account the sign of the response, these phase shifts are in accordance with the phasic character of the unit. The green phase characteristics are always steeper than the red ones. This general phenomenon suggests that the latency of a green coded response always exceeds the latency of the red component when red and green stimulus intensities do not differ too much. The latency difference is of the order of 15–40 msec.
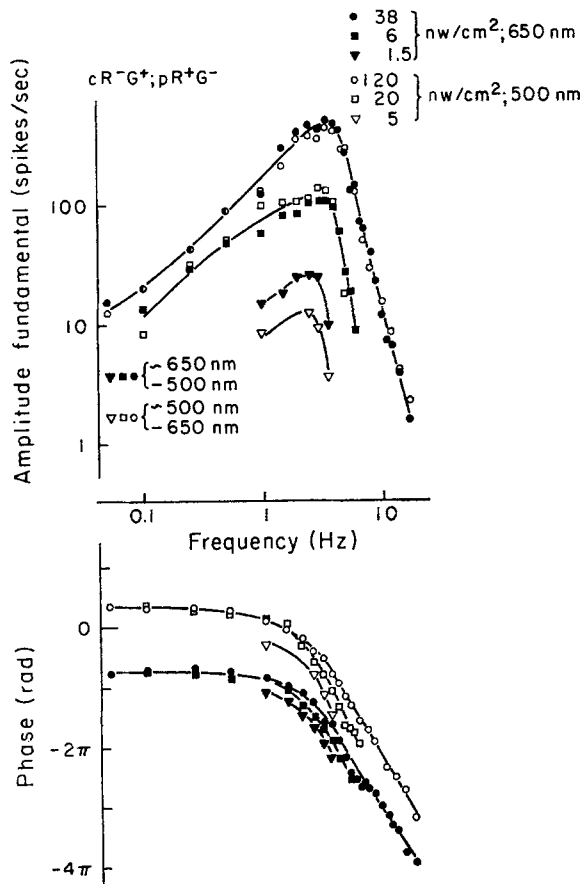
FIGURE 5

FIGURE 6

FIGURE 5. Amplitude and phase characteristics for each of the two spectrally coded central processes of a silent ganglion cell are depicted as a function of mean intensity. The superimposed red (650 nm) and green (500 nm) stimulus spots with a diameter of 1 mm were always presented simultaneously, but in each experiment only one of the two was modulated sinusoidally. Modulation depth is 20% for the highest intensities and 50% for the lower intensities. The amplitudes are normalized to 100% modulation depth. The temperature of the isolated retina is 14.5°C.

FIGURE 6. Amplitude and phase characteristics of the central green process of a silent ganglion cell to a spot of a Gaussian noise–modulated ($\sigma = 40\%$) light of constant mean intensity and different spot sizes. The mean intensity of the stimulus after passing through a broadband 540 nm interference filter is 550 nw/cm². The temperature of the preparation is 16.7°C.

5. *Dynamic Characteristics as a Function of Spot Diameter*

Fig. 6 gives the amplitude and phase characteristics determined with a monochromatic Gaussian noise stimulus for two spot sizes.

These characteristics are obtained from the green central process of a
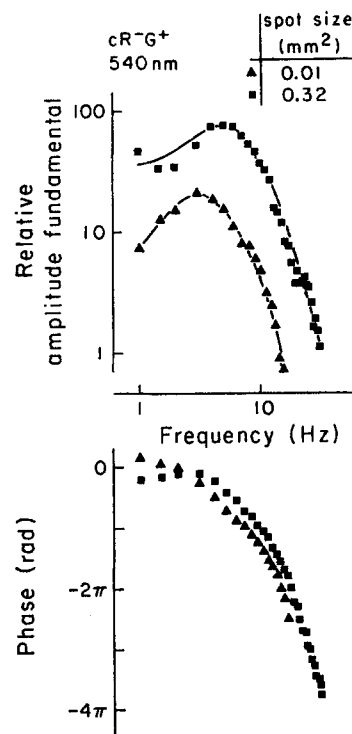
silent phasic ganglion cell. They illustrate that for a given intensity an increase in the diameter of the stimulus spot shifts the cut-off frequency to higher values and increases the sensitivity of the amplitude characteristic. This holds for spot diameters up to approximately 1 mm on the retina. Comparison of these data with the data in Fig. 4, which were obtained from another ganglion cell, suggests that it is not the intensity itself but rather the energy per unit of time (power) falling within the center of a receptive field that determines the shape of the amplitude characteristic. This means that the shape of the amplitude characteristic as a function of intensity is not determined in one of the early retinal processes (for example the photoreceptors) because there is marked spatial summation. Such summation is not found in the most distal retinal stages.

6. Dynamic Characteristics of Central and Peripheral Processes

The dynamic charcteristics of the central and peripheral processes can be compared legitimately only if both the relative sensitivities of the spatial coded processes and the sizes of the stimulated areas are taken into account. This follows from the data in Figs. 5 and 6.

Fig. 7 gives the amplitude characteristics of the various components of a spontaneous ganglion cell. Both red-off and green-on central processes contributed to the response of this particular cell, whereas in the periphery only a red-on component was present. Since the sensitivities of the various components have to be taken into account, the amplitude and phase characteristics are presented at three stimulus intensities. An intensity can always be found for which the amplitude characteristics are no longer distinguishable with respect to the spectral and spatial coding of the response component. If the intensities are chosen such that the amplitude characteristics are identical irrespective of the spectral and spatial coding of the response components, then a change in intensity by the same factor results again in an identical set of amplitude characteristics. This follows directly from the drawn amplitude characteristics in Fig. 7. A reduction in intensity by a factor of 10 gives again an identical set of amplitude characteristics. However, even in these matched conditions the phase characteristic of the peripheral process is still steeper than the phase characteristic of the central processes. This indicates that the peripheral process has a longer latency. The latency difference between central and peripheral processes is generally of the order of 50 msec.

DISCUSSION

The goal of this paper was to determine whether the dynamic properties of the retinal ganglion cell responses in goldfish could be distinguished with respect to their spectral and spatial coding. The results indicate that under

appropriate conditions the amplitude characteristics of the central and peripheral processes are identical, irrespective of the sign and the spectral coding of the response. On the other hand, even with identical amplitude characteristics for central and peripheral processes, the phase characteristics
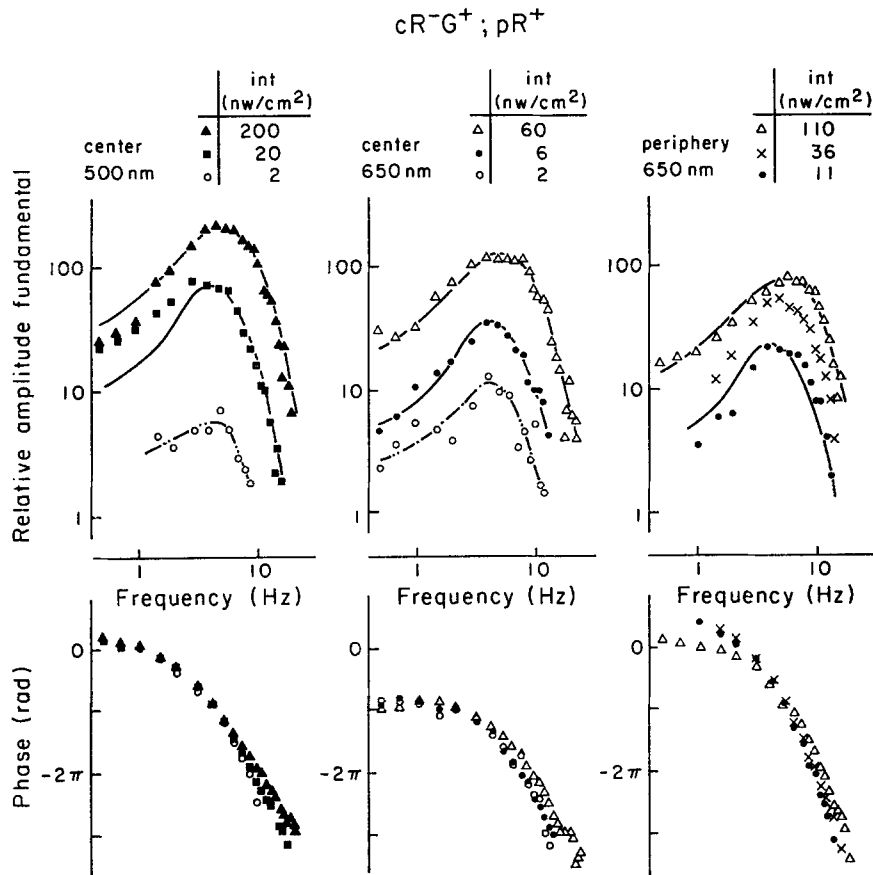

FIGURE 7. Amplitude and phase characteristics for each of the two central (red-off, green-on) processes and for the peripheral (red-on) process of a spontaneous ganglion cell as a function of the mean intensity of a Gaussian noise–modulated (σ = 40%) monochromatic stimulus light. The central stimulus spot has a diameter of 1 mm; the peripheral anulus has an inner diameter of 2.5 mm and an outer diameter of 4 mm. The temperature of the isolated retina is kept at 16.1°C.

still differ. The enhanced steepness of the phase characteristic for the peripheral process indicates a longer latency. This latency difference might be explained to some extent by taking into account the longer distances in the plane of the retina which the peripheral signals must travel before they reach the ganglion cells.

The identity of the amplitude characteristics of center and periphery indicates that both synaptic pathways to the ganglion cell have the same amplitude characteristics. Therefore we have no evidence for interneurons unique to the periphery of receptive fields in goldfish. Such interneurons have been reported by Naka (31) for catfish and are suggested by Dowling
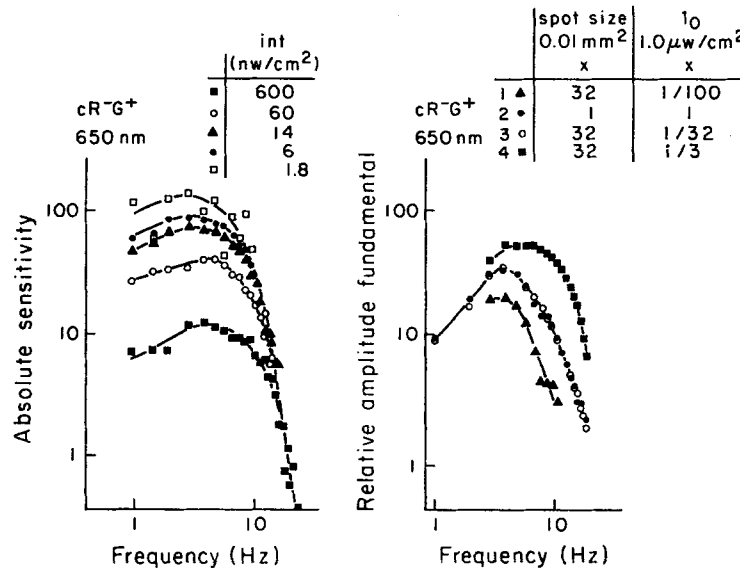

Figure 8. In the left column the data of the spontaneous ganglion cell of Fig. 4 are replotted with the vertical axis in absolute sensitivity. The data points for high frequencies join together in a common envelope. In the right column the amplitude characteristics of the central red process of a spontaneous bichromatic ganglion cell are depicted as a function of the energy per unit of time (power) of a Gaussian noise–modulated (σ = 40%) stimulus light. For spot diameters of up to about 1 mm, a constant energy per unit of time E gives identical (curves *2* and *3*) amplitude characteristics ($E = A \times I_o$, where A is the stimulus area and I_o is the mean stimulus intensity after passing through a 650 nm broadband interference filter). For a fixed diameter of the stimulus spot the sensitivity and shape of the amplitude characteristics are determined by intensity (curves *1*, *3*, and *4*). The temperature of the preparation is 17.1°C; the signal samples are 9 min.

and Boycott (12) on the basis of histological data for the primate retina. Maffei et al. (28) interpreted their data on ganglion cell responses in cat retina in this fashion also. They found a lower cut-off frequency for the peripheral process than for the central process. However, their data do not offer firm support for Dowling and Boycott's hypothesis because they did not examine the influence of stimulus area and intensity upon the shape of the amplitude characteristic. In the present paper we have shown that these parameters determine to a large extent the shape of the amplitude characteristics in the goldfish retina.

Many models (14, 26, 29, 42) have been proposed to account for the shift to higher values of the cut-off frequency in the amplitude characteristic as the mean stimulus intensity is increased. It has been shown both for psychophysical (19, 26) and for electrophysiological data (22, 40) that an envelope can be obtained by plotting the absolute amplitude rather than the modulation depth as a function of frequency. The left column of Fig. 8 gives the data of the spontaneous ganglion cell of Fig. 4 with the vertical axis replotted in absolute sensitivity.[7] The data points for high frequencies join together in a common envelope. Such an envelope can be found for all spontaneous cells. Anywhere along the envelope the high frequency response depends on the absolute and *not* on the relative modulation depth, in accordance with the well-known Ferry-Porter law. In addition to the high frequency attenuation, which expresses itself in the common envelope, there is a low frequency attenuation which increases with intensity. The shape of the envelope is identical for central and peripheral processes, irrespective of the sign and the spectral coding of these processes. However, the envelope holds only over a restricted range of intensity (three log units). For the highest stimulus intensities, the amplitude characteristics deviate from the common envelope due to an over-all reduction in sensitivity. For still higher intensities the response strength may even decrease with increasing intensity, as can be seen for the silent ganglion cell of Fig. 4. It should be noted that no common envelope can be obtained for silent ganglion cells. Silent cells exhibit a threshold effect which is stronger the lower the mean intensity. This follows directly from the data in the left column of Fig. 4. For low intensities doubling the intensity leads to a disproportionate increase in response strength.

The low frequency ends of the amplitude characteristics seem to depend on mean intensity. However, the right column of Fig. 8 shows that the power falling within a receptive field rather than the intensity determines the location and the shape of the amplitude characteristic. In this figure the same amplitude characteristic can be obtained by increasing the stimulated retinal area by a factor of 32 and diminishing the intensity by the same factor or vice versa (curves 2 and 3). This reciprocity of area and intensity holds only for spot diameters up to about 1 mm, consistent with the diameter of the full summation field in goldfish (41). Therefore, the above data indicate that the diameter of the full summation area remains the same for threshold and suprathreshold measurements.

In conclusion, the present findings demonstrate that receptor processes cannot be sole determinants of the shapes of the amplitude characteristics because spatial summation plays such an important role. Although spatial

[7] This means that the curves of Fig. 4 are normalized to the absolute (σ I) and not to the relative (σ) stimulus strength.

summation has been reported for cones in the retina of turtle (1), significant spatial summation has not been found at the level of the receptors in fish retina (43). Furthermore, Kaneko (17) has shown that the bipolar cell responses in fish exhibit an antagonistic spatial organization. If the receptive fields of the ganglion cells are already completely determined at this retinal stage, then the dynamic characteristics of the ganglion cell responses might be mainly formed in the retinal layer preceding the ganglion cells. However, our experiments do not allow for an evaluation of the contribution of the ganglion cells to the final form of their dynamic characteristics, since an algebraic mode of operation governs the spectral and spatial interaction in the goldfish retina (39). With such a linear interaction it is impossible to localize the sequence of the various retinal transformations. Only a direct analysis of the intervening stages, such as the bipolar cells, can lead to a more precise location of the mechanism which determines the dynamic characteristics of spike discharges as a function of stimulus power.

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