The Dynamic Characteristics of Color-Coded S-Potentials

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ABSTRACT A linear analysis approach has been applied to determine the dynamic characteristics of the color-coded S-potentials. Using a sinusoidally modulated light stimulus it could be shown that the monophasic S-potential as well as each of the different components of biphasic and triphasic S-potentials behaves linearly. However, for high modulation depths and high average intensities nonlinear effects, such as saturation, become obvious. The transfer characteristics of the monophasic potentials and each component of the biphasic and triphasic potentials are indistinguishable. Their latencies, however, differ. These findings suggest that the three different types of S-potentials not only originate from functionally comparable cells but also that the dynamic characteristics of the cells presynaptic to the S-potential sources are identical.

In recent years much attention has been given to a curious type of graded response that has been found in the retinas of many vertebrates. These responses were described for the first time by Svaetichin (8) for the teleostean retina. The origin of these potentials is a point of controversy and in order to provide them with a biologically nonspecific name they have been named after Svaetichin and are called S-potentials. It can be said with certainty only that the S-potentials originate in the layers that lie between the photoreceptors and ganglion cells in the retina (1, 5, 9, 11).

In the fish retina, three fundamentally different types of spectral response curves have been observed for the S-potentials. One type, the monophasic S-potential, is hyperpolarizing to all wavelengths between 400 and 750 nm. Another type of S-potential, the biphasic response, is hyperpolarizing to green and depolarizing to red stimuli. The third type of S-potential is a triphasic response, which consists of components that are depolarizing to green and hyperpolarizing to blue and red portions of the spectrum (6, 7).

In a previous paper (7) it was demonstrated for the carp retina that the three S-potential types have identical spatial organizations, and therefore
could not be differentiated on that basis. In the present study, we investigate
whether they may be distinguished by their dynamic characteristics.

Analysis Technique

A linear approach has been chosen to determine the dynamic characteristics
of the S-potentials. It may be argued that such an approach is unjustified be-
cause many of the psychophysical relations between stimulus intensity and
response are nonlinear. Also, electrophysiologic experiments have revealed
nonlinearities such as power relationships and logarithmic transformations at
different retinal stages of signal processing (3, 4). However, for the analysis of
the color-coded S-potentials a linear approach proved to be appropriate. Ex-
amples will be shown of graded responses which duplicate closely the wave-
form of sinusoidal stimuli, whose amplitudes are small in comparison to the
dynamic range of the S-potential source. Furthermore, a sine wave is chosen
as the primary stimulus since a sine wave passes undistorted through a linear
system; therefore, any distortion in the response (harmonics) reveals directly
the range over which the linear analysis holds (2). This is not so for any other
type of periodic stimulus, since these contain harmonics whose amplitudes and
phases may be changed after transmission through a linear system (linear
distortion). Therefore, nonlinear distortions in the response are not as easily
detected as with a sinusoidal stimulus.

In a linear system a sinusoidal input $I(t) = A \sin 2\pi ft$, results in a sinusoidal
response $R(t) = B \sin(2\pi ft + \phi)$. Thus a linear system affects only the amplitu-
de and phase of a sinusoidal input signal. Therefore any linear system is
fully described by two relations: the amplitude ratio $T(f)$ of the input and
output signal as a function of the stimulus frequency, $T(f) = \frac{B(f)}{A(f)}$, and sec-

Figure 1. The intensity of a sinusoidal light stimulus is depicted as a function of time.
The unmodulated stimulus of intensity $L_o$ changes at a given moment into a sinusoidal
modulated light with amplitude $A$. The depth of modulation is conveniently expressed
by the parameter $m = A / L_o$. The stimulus frequency is $f$ cps. The mathematical repre-
sentation of the waveform is indicated in the figure.
ond, the phase shift $\phi(f)$ between input and output as a function of frequency. The first relationship is known as the amplitude characteristic and the second as the phase characteristic. The mathematical expression relating these two characteristics is the transfer function of the linear system.

Since neither the amplitude characteristic nor the phase characteristic of the linear system is dependent upon the amplitude of the input sine wave, the transfer function based on experimental measurements allows us to predict the response for any other type of input signal. This is the advantage of a linear system approach.

In Fig. 1 the intensity of a sinusoidal stimulus is depicted as a function of time. At a given moment, the steady light with an intensity $L_0$ changes into a sinusoidally modulated light with a frequency of $f$ cycles/sec. The degree of modulation is conveniently expressed as a dimensionless parameter, $m = A/L_0$, in which $A$ is the amplitude of the sine wave. The parameter $m$ varies between 0 (0%) for unmodulated and 1 (100%) for fully modulated light. Notice that the average intensity $L_0$ remains the same for all values of $m$ and $f$.

![Figure 2](image)

**Figure 2.** Three common types of S-potentials: monophasic (a), biphasic (b), and triphasic (c). In all records, a negative potential deflection (hyperpolarizing) at the recording electrode is downward. Horizontal traces—marked *resting potential*—indicate the potential level in the absence of a light stimulus. Direction and duration of the spectral scan for each record are indicated by arrow at top. Intensity of the stimulus at 650 nm is 9 $\mu$W/cm². For the energy spectrum of the light stimulus see reference 10. The records were made with a pen recorder.
METHODS

Preparation. Whole retinas were dissected from freshly enucleated eyes of carp, Cyprinus carpio. The retina was placed, receptor side up, in a water-cooled chamber. The light stimulus was projected from below, passing from ganglion cell to photoreceptor layer. The chamber was kept at 16°C and had moist oxygen passing through it. The recording electrodes were glass micropipettes filled with 3 M KCl solution. The pipettes had a resistance of the order of 200 megohms and were selected for low noise levels. Under direct microscopic observation the micropipette was centered with respect to the stimulus field.

Optical Stimulator. The two-channel optical stimulator is a modification of one previously described (10). One channel utilizes the light beam from a tungsten projection lamp. The beam passes successively through a monochromator and a neutral density wedge. The other channel has a light source consisting of electronically controlled fluorescent lamps (F 15T8 cool white) to generate periodic waveforms. A photocell is used to monitor the different stimulus waveforms. The spectral characteristics of the second channel are determined by interference and Wratten filters.
Neutral density filters controlled the stimulus intensity. In both channels, different stimulus configurations such as spots and annuli could be focused upon the retina.

**Recording** Signals from the recording micropipettes were fed to a high input impedance, capacitance compensated DC amplifier via Ag/AgCl electrodes. The cutoff frequency of the system was approximately 5 kc, well above the highest frequencies found in S-potentials. During an experiment the microelectrode was slowly advanced through the retina by a micromanipulator. In order to facilitate the isolation and classification of a unit, the wavelength of the stimulus was continuously varied from 400 to 750 nm. Once a unit was isolated the optical stimulator was switched over to the sinusoidally modulated light channel for determining the dynamic properties of a particular component.

**Data Analysis** The output of the amplifier was displayed on an oscilloscope. This signal and the synchronized trigger pulses from the function generator controlling
the modulated light source were fed to a tape recorder. In order to improve the signal-to-noise ratio the responses to all periodic stimuli were analyzed off-line by means of a computer of average transients. The number of counts, or averages, was generally 20 for low frequency responses, and 200 for frequency responses of 10 cps or more. The taped output of the stimulus-monitoring photocell was also fed to one of the computer channels so that the average responses could be compared with the
summed stimulus signal. This was done in order to reveal any defects (distortion) in the stimulus and to facilitate measurement of amplitude and phase characteristics. A final record for computations was obtained by plotting the analogue output of the computer with an x-y recorder.

RESULTS

Three types of color-coded S-potentials were isolated. An example of each is given in Fig. 2.

Fig. 2 A is representative of the monophasic S-potentials. This purely hyperpolarizing response shows the largest sensitivity in the region of 640 nm. Fig. 2 B illustrates a commonly found type of biphasic S-potential. The maximum sensitivity of the hyperpolarizing green component is near 530 nm, whereas the depolarizing red component peaks around 670 nm. The third type of S-potential is a triphasic response which consists of a depolarizing green and hyperpolarizing blue and red components. The maximum sensitivity for each component is near 470 nm for blue, 560 nm for green, and 670 nm for red (Fig. 2 C).

Once a particular type of S-potential was isolated, the wavelengths which gave the largest amplitude responses were chosen to determine the dynamic properties of the depolarizing and hyperpolarizing components of the three types of S-potentials.

Linear vs. Nonlinear

As pointed out in the introduction, linear systems are characterized by two properties: (a) a sine wave passes undistorted through a linear system; (b)
FIGURE 6. Fig. 6 A gives the averaged phase characteristic for the monophasic, Fig. 6 B for the two components of the biphasic, and Fig. 6 C for the three components of the triphasic S-potentials. These phase characteristics correspond to the amplitude characteristics of Fig. 5. All the depolarizing components exhibited an initial phase shift of zero degrees. However, the blue component of the triphasic S-potential was displaced 360 degrees to prevent overlapping of the curves.
the amplitude of the response is proportional to the amplitude of the stimulus.

The first property is demonstrated for a monophasic unit in Fig. 3 A; the responses to square wave (3 B) and triangularly modulated (3 C) light are also presented. As shown, the response waveforms duplicate the stimulus waveforms for moderate modulation depths and low stimulus frequencies. For higher stimulus frequencies (> 10 cps) all responses are found to become sinusoidal and less dependent on the stimulus waveform. This indicates a high-frequency cutoff in or preceding the S-potential compartment. Furthermore, for high modulation depths and high average intensities, distortions such as saturation become obvious in the response.

The second property provides another experimental criterion for linearity. In Fig. 4 the amplitudes of monophasic S-potentials are plotted for different intensities as a function of the modulation depth.

These curves show: (a) For each intensity used, the amplitude of the response is proportional to the modulation depth. (b) For a chosen modulation depth, the amplitude of the response is proportional to the intensity. This means that the amplitude of the response is proportional to the absolute amplitude, \( m_Lo \), of the stimulus.
Similar results are obtained for each of the components of biphasic and triphasic S-potentials. Because of the above, we feel it to be justifiable to apply a linear analysis technique to determine the dynamic characteristics of the S-potentials.

Amplitude and Phase Characteristics

The average amplitude characteristics for monophasic S-potentials, and for each of the components of biphasic and triphasic S-potentials, are given in Fig. 5.

The vertical axis of the amplitude characteristic is conveniently expressed in decibels (i.e. 20 log $T(f)$) and the horizontal axis in log $f$. It is evident from these curves that the amplitude characteristics are identical for these types of S-potentials and their components.

The phase characteristics which correspond to the amplitude characteristics shown in Fig. 5 are plotted in Fig. 6.

For low stimulus frequencies (< 1 cps) the phase characteristics are identical, except for a phase shift of 180° due to the opposite sign of hyper- and depolarizing responses. For higher stimulus frequencies, the shapes of the hyperpolarizing monophasic S-potential, the hyperpolarizing green component of the biphasic S-potential, and the hyperpolarizing blue component of the triphasic S-potential are identical. The phase characteristics of the depolarizing components in biphasic and triphasic responses are also identical to each other but are steeper than the above-mentioned hyperpolarizing components. The hyperpolarizing red component of the triphasic response exceeds all other components in phase shift.

Intensity Dependence

In Fig. 7 B the amplitude characteristics of monophasic units, measured at three different intensity levels, are plotted. Since the adaptation levels are $L_0$, $\frac{1}{2} L_0$, $\frac{1}{4} L_0$, the curves are displaced 6 dB (i.e. 20 log 2) with regard to each other. The shape of the curves is independent of the average intensity, over the intensities investigated.

The same holds for the corresponding phase characteristics that are shown in Fig. 7 A.

Latency

A latency expresses itself as a phase shift between the response and the sinusoidal stimulus. The relationship between the phase lag $\phi$ in radians, the sine wave stimulus frequency $f$ in cps, and the latency $\tau$ in sec is given by $\phi = 2\pi f \tau$. For a fixed stimulus frequency the latency change as a function of the adaptation level is $\Delta \tau = \Delta \phi / 2\pi f$. In order to obtain a high accuracy, a high frequency $f$ should be chosen. The choice of the stimulus frequency $f$ is, how-
ever, restricted by the cutoff frequency of the S-potential source. For the chosen stimulus frequency of 8 cps, \( \Delta \tau \approx (1/3) \Delta \phi^o \) (msec). The latency changes determined in this way for the three S-potential types are plotted as a function of the average intensity in Fig. 8.

It may be concluded from these data that over the measured intensity range of 1.8 log units the latencies of the monophasic potential, the green component of the biphasic potential, and the blue component of the triphasic potential are independent of the average intensity. Once again these three particular responses behave similarly. For the red component in the biphasic S-potential as well as for the green and red components of the triphasic potential, the latency appears to increase with decreasing average intensities.

**Spatial Interaction**

Fig. 9 shows the average latency change of the monophasic potential as a function of the distance between stimulus spot and center of receptive field. It can be calculated from the slopes of the curve in Fig. 9 A that the propagation velocity in the S-potential layer of the retina is 0.3 m/sec.
Figure 8. The latency changes for monophasic responses and for each of the components of biphasic and triphasic S-potentials are plotted as a function of the average stimulus intensity. The latencies were derived from the phase shifts of the responses to a sinusoidal stimulus of 8 cps. The average intensity of the stimulus was varied over a range 1.8 log units. At zero log attenuation the average intensity approximates 1 µw/cm².
Fig. 9 A demonstrates that the amplitude of the monophasic response decreases with the distance from stimulus spot to electrode position.

**DISCUSSION**

In a linear, minimum phase-shift system the amplitude and phase characteristics are directly related to each other. This means that in such a system the amplitude characteristic determines the shape of the phase characteristic and vice versa. We used the amplitude characteristics given in Fig. 6 to calculate the transfer function of the system: light input, S-potential output.

In Laplace notation, a transfer function has the form $T(s) = \frac{N(s)}{D(s)}$. The roots of the numerator are called the zeros and the roots of the denominator are the poles of the system. Each zero accounts for a low frequency cutoff of 6 dB/octave and each pole for a high frequency attenuation of 6 dB/octave. (An octave, being the difference between a frequency $2f$ and $f$, is represented by a constant distance on a logarithmic scale.)

The amplitude characteristics of Fig. 5 show (a) there is no decrease in sensitivity for stimulus frequencies below 3 cps; (b) for stimulus frequencies above 15 cps the sensitivity of the system decreases with a constant 24 dB/octave. Therefore a transfer function with no zeros and four poles is sufficient to describe the amplitude characteristics. The values of the poles are determined by the best fit of the calculated amplitude characteristic, with the
actual data points between 3 and 15 cps (see Fig. 5). The transfer function:

\[ T(s) = \frac{\omega_0^4}{\left(s^2 + \frac{\omega_0}{Q} s + \omega_0^2\right)^2}, \]

with cutoff frequency \( \omega_0 = 2\pi \times 11.5 \) and \( Q = 0.7 \), gives the amplitude characteristics shown in solid lines in Fig. 5.

The maximum phase shift due to a single pole amounts to \( \frac{\pi}{2} \) radians. The above transfer function can therefore only account for a phase shift of \( 4 \times \left(\frac{\pi}{2}\right) = 2\pi \) radians. Since the difference between the calculated and the measured phase shift proved to be directly proportional to the frequency, a delay time can account for the excessive phase shift observed in Fig. 6.

With the formula \( \tau = \Delta\phi / 2\pi\Delta f \), a latency of 23 msec has been calculated for the monophasic potential, the green component of the biphasic, and the blue component of the triphasic S-potential. The latencies for the red component of the biphasic and the green component of the triphasic S-potential are also identical and amount to 55 msec. The red component of the triphasic S-potential has the longest delay, with a latency of 74 msec.

**SUMMARY**

1. The experiments show that the three different types of S-potentials behave linearly.
2. The amplitude characteristics of the monophasic potentials and all the components of biphasic and triphasic S-potentials are identical. The high-frequency attenuation amounts to 24 dB/octave and the cutoff frequency lies near 11.5 cps.
3. The latencies of monophasic S-potentials, the green component of the biphasic, and the blue component of the triphasic S-potentials are identical. The latency is of the order of 25 msec and is independent of the average intensity.
4. The latencies of the red component of the biphasic and the green component of the triphasic S-potentials are identical and of the order of 50 msec. This latency is a function of the average stimulus intensity.
5. The latency of the red component of the triphasic S-potential is of the order of 75 msec and is also dependent upon the stimulus intensity.
6. The propagation velocity in the retina as determined for monophasic S-potentials amounts to 0.3 m/sec.

From these results the conclusion can be drawn that the three types of color-coded S-potentials exhibit the same dynamic behavior. Therefore it is evident that the monophasic, biphasic, and triphasic S-potentials originate from functionally similar cells. Recent anatomical studies on carp retinas have revealed three distinct layers of horizontal cells (12). Cells in these layer seem likely candidates to be the source of the S-potentials.
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