TEMPORAL ANALYSIS OF VISUAL RESPONSES FROM THE PROG RETINA

A Thesis submitted for the degree of Ph.D.

by

KENNETH ALLAN FRANCIS GRATION B.Sc.

Department of Physiology, School of Biological Sciences,
University of Leicester.

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This is to certify that the thesis I have submitted in fulfilment of the requirements governing candidates for the degree of Doctor of Philosophy in the University of Leicester, entitled "Temporal Analysis of Visual Responses from the Frog Retina", is the result of work done primarily by myself during the period of registration for the above degree.

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INTRODUCTION.
The changes in retinal sensitivity associated with light or dark adaptation are accompanied by changes in the temporal characteristics of the retina. In the light adapted Limulus eye, the receptor potential in response to a unit input reaches a maximum before a similar potential evoked by the same input under dark adapted conditions (Hartline and McDonald, 1947; Hartline, Wagner and MacNichol, 1952; Fuortes and Hodgkin, 1964). In this preparation, sensitivity and time resolution vary in a reciprocal manner. A similar relationship exists in the vertebrate retina as shown by Cone and Matt, (1964). They found that the b-wave of the rat E.R.G., in response to step inputs, in constant ratio to a number of different steady background intensities, is displaced on the time axis. In the rat the latency of the response is shortened by an increase in the absolute background intensity. As increased background intensity results in decreased sensitivity, these results indicate that the latency of the b-wave is shortened as sensitivity is decreased.

Of the numerous studies, which have been made upon the changes in sensitivity of visual systems, few have approached the problem of the reciprocal relationship between sensitivity and temporal resolution. Any model which attempts to describe retinal function in mechanistic terms must explain this relationship.

An understanding of the processes involved in the recovery of visual information has been derived first from the study of the input-output relationship for visual thresholds, and second from the electrophysiology of specific neurones e.g. receptors, bipolars, ganglion cells etc. These two methods of study define the characteristics of the mechanisms involved in information processing.
Knowing these characteristics, a number of models have been proposed to explain visual function in mechanistic terms. Rushton (1962) proposes an automatic gain control mechanism to explain sensitivity changes in the human retina, and Fuortes and Hodgkin (1964) describe the generation of the receptor potential in Limulus in terms of a linear filter model. These two mechanistic approaches to visual function are based upon different experimental approaches:

(1) The study of threshold changes during dark adaptation.

(2) Transient and frequency analysis (Appendix).
Models based upon the study of threshold changes during dark adaptation.

Changes in sensitivity during dark adaptation have been measured using both psychophysical and electrophysiological techniques. Psychophysical determinations of threshold for extra-foveal vision in the human eye show that a progressive fall in threshold is evident when a light adapted subject is placed in total darkness. The time course of these threshold changes is not smooth, but shows a "kink" which separates two phases of adaptation. An initial rapid phase of increasing sensitivity, which is complete after five to ten minutes, is separated from a second slower phase by a plateau of constant sensitivity (Arden and Weale, 1954). Electrophysiological determinations of the threshold changes during dark adaptation, observed when recording from single ganglion cells in the frog retina, confirm these observations (Parlow, 1953a; Boumann and Scheibner, 1968). The interpretation of the shape of dark adaptation curves, in terms of the Duplicity Theory of vision, is that two separate mechanisms of vision exist. First, photopic vision which functions at high light intensities and is cone mediated, and second scotopic vision which functions at low intensities and is rod mediated. The transition from photopic to scotopic vision is gradual as dark adaptation proceeds indicating that an intermediary range of intensities exists over which both mechanisms operate together: the mesopic range.

Two hypotheses are proposed to explain changes in retinal sensitivity and their dependence upon dark adaptation and background intensity:

A). The Receptor-Desensitisation hypothesis.
B). The Bleaching Signal hypothesis.

As the time course of rhodopsin regeneration roughly corresponds with that of dark adaptation, it was suggested that changes in sensitivity during dark adaptation are related to regeneration of visual pigment in a
simple way (Crawford, 1946). This simple view supports the photochemical theory of vision, in which threshold is assumed to be inversely proportional to the amount of photopigment present in the retina. This theory, however, does not account for the logarithmic relationship between the intensity required to evoke a constant amplitude b-wave in the rat E.R.G. and the concentration of free opsin (bleached rhodopsin) (Dowling, 1960).

A logarithmic relationship clearly disproves any theory which attempts to explain changes in retinal sensitivity in terms of simple receptor desensitisation. To circumvent this objection to the receptor desensitisation hypothesis, a compartment hypothesis is proposed (Wald, 1954). This is based upon the microstructure of rods, as revealed by electron microscopy, which shows that the rhodopsin in each rod is compartmentalised into anatomically distinct structures. From these observations Wald suggests a mechanism to explain sensitivity changes by which any compartment becomes wholly refractory whenever one or more molecules of rhodopsin within it are bleached. The compartment then remains unexcitable until every molecule is regenerated. Large changes in sensitivity can therefore occur for only small bleaches, because the absorption of a single light quantum can make a whole compartment refractory. This model predicts that for small bleaches log threshold is proportional to the concentration of bleached rhodopsin.

A further prediction of the model is that refractory compartments are inert. Because of this inert nature, the compartment hypothesis cannot account for two features of retinal responses during dark adaptation. First, that threshold changes depend upon the area of the light stimulus used to evoke the test response (Craik and Vernon, 1941; Arden and Weale, 1954). And second, when using large fields the rate of change of threshold is faster than when small fields are used. These observations are interpreted as indicating increased area...
summation at low intensities. This interpretation is confirmed by the receptive field studies of Barlow et al. (1957). They observed that in the cat retina the receptive field organisation of ganglion cells changes during adaptation. The fields of cells giving pure "on" responses in the dark adapted state increase in size when light adapted to produce complex "on" centre and "off" surround fields. In the light adapted state, therefore, the "on" centre response is antagonised by lateral inhibition from the surround. During dark adaptation the neural networks of the retina reorganise to leave the pure "on" centre response. The change in organisation is not linked to the Purkinje shift, and measurements of the area-threshold relationship for red and blue stimuli show that it is not dependent upon a change from cone to rod mediated vision. It is therefore suggested that the change in organisation results from a disappearance of the lateral connections which mediate surround inhibition. These observations are inconsistent with the compartment hypothesis, because as refractory compartments are inert they cannot affect the neural reorganisation of the retina seen during adaptation.

A second observation inconsistent with the compartment hypothesis is that of rod-rod interactions in the human retina (Rushton and Westheimer, 1962). A bright adapting flash was projected onto the retina, through a grating in front of the eye. The retina was therefore exposed to alternate light and dark strips. The rods exposed to the adapting flash exhibited increased thresholds because they were light adapted. The thresholds of the rods in the dark regions were also increased even though the rods were not exposed to the adapting flash. This observation shows that bleached rods affect the thresholds of their unbleached neighbours. This is inconsistent with the inert nature of the refractory compartments proposed by the compartment hypothesis, because an adapting light falling upon a restricted region of the retina should not affect the sensitivity of neighbouring rods.
Taken together these observations, by Craik and Vernon, (1941); Barlow et al, (1957); and Rushton and Westheimer, (1962), disprove any hypothesis based exclusively upon receptor desensitisation. Two separate interpretations of the causal relationships between sensitivity and dark adaptation or background illumination are offered by Barlow, (1964) and Rushton, (1965c). Both interpretations suggest that changes in sensitivity are attributable to changes in the intrinsic noise level of the eye, rather than to changes in the attenuation of light evoked signals. The simplest interpretation is proposed by Barlow who suggests that the signals from partly bleached receptors are identical to those evoked by light. Barlow's hypothesis is based upon the assumption that bleached rods transmit their signals with normal sensitivity when stimulated, but that they also transmit signals in proportion to the amount of bleaching. Sensitivity is therefore a function of the signal to noise ratio, which must be high to exceed threshold. In the dark adapted state, when receptor noise is minimal, a just detectable stimulus will promote a response which satisfies the high ratio criterion. When the eye is partially light adapted however, signals arising from bleached rhodopsin increase the noise level. The stimulus, which was previously detected, now gives rise to a response with a signal-noise ratio below threshold. This represents a decrease in the sensitivity of the eye. In addition to the control of sensitivity, Barlow suggests that the signals from bleached receptors also control neural organisation. This suggestion explains the similar time-course of spatial summation and small field adaptation during dark adaptation (Blakemore and Rushton, 1965).

Rushton, (1962; 1965c) proposes a different hypothesis to explain the function of bleaching signals in the control of sensitivity. This assumes that bleaching signals act directly upon an automatic gain control mechanism at a level distant from the receptors (Rushton's summation pool). Signals derived from the illumination of receptors,
however, are only assumed to have an indirect effect upon gain, which results from feedback. In formulating such a hypothesis Rushton suggests that the signals which result from bleaching and those caused by illumination of the receptors are different. This suggestion is difficult to interpret, because the signals arising from both bleaching and illumination are a result of rhodopsin breakdown.
Models based upon transient and frequency analysis.

Both transient and frequency analyses are only applicable to systems whose responses are a linear function of the input (Appendix). Devoe,(1962;1963), when recording retinal action potentials from the eye of the Wolf spider, Lycosa, found both linear and non-linear responses. He demonstrated that the linear responses were evoked from the eye when stimulus amplitudes were restricted to those which gave only small (10 - 20μV peak-peak) responses. Following this study numerous authors showed that it is possible to obtain satisfactorily linear responses from visual systems by restriction of the stimulus conditions so that only a small range of the systems non-linear characteristics is traversed (Brindley and Westheimer,1968; Pinter, 1966; Schellart and Spekreijse,1972).

It is advantageous to restrict the responses to a linear range because both transient and frequency analysis describe the sensitivity and time dependent parameters of the system. Either of these methods proves to be a more powerful analytical tool than threshold determinations which only measure sensitivity. While acknowledging the advantages of both methods of analysis, it must be accepted that while restricting a non-linear system to a linear response range, no analysis -is can describe every aspect of the systems function. It does, however, provide a useful means of approach to, and an understanding of, the systems parameters.

Transient generator potentials recorded from the retinular cells of Limulus show that the time scale of the responses shortens as the eye is light adapted (Hartline and McDonald,1947; Hartline, Warner and MacNichol,1952). Fuortes and Hodgkin(1964) analysed both impulse and step responses when studying the quantitative relationships between the changes in time scale and sensitivity. They found that weak stimuli evoke linear responses, which when analysed in terms of a linear filter hypothesis, can be fitted by an equation containing ten stages.
of exponential decay whose time constants are equal. When the
stimulus intensity is increased, the initial rising phase of the
transient remains linear but the later parts become non-linear.

Over the linear range of responses a quantitative relationship
between sensitivity and time scale exists, such that a two hundred fold
reduction in sensitivity is associated with a halving of the time con-
stants of the linear filter. As retinular cell sensitivity and the
time constants are proportional an automatic gain control, incorporating
feedback, is proposed to explain the relationship. This type of
mechanism reduces sensitivity at high intensities and at the same time
shortens the time constants of the filter. Gain control is assumed to
be mediated by the output (a feedback mechanism) rather than the input,
because this is consistent with the observation that, at very early
times, depolarisation is proportional to light intensity. Only later
does the relationship become logarithmic.

Equations incorporating feedback are found to fit the main features
of the visual responses of Limulus ommatidia over a wide range of
intensities. The use of a linear filter hypothesis, which is capable of
predicting the visual responses, does not assume that any structure
equivalent to a cascade of ten filter elements exists in Limulus
ommatidia. However, in relating sensitivity and time resolution by a
feedback control, it does suggest a tentative hypothesis to explain
their interrelationship.

Although transient analysis reveals a considerable amount of
information concerning the dynamic nature of Limulus visual responses,
this technique is not as widely used as frequency analysis. The
difficulty encountered with transient analysis is the measurement of
time constants from the system's responses. This difficulty is overcome
when a frequency analysis is performed. The frequency response curves
of the system's gain, derived when implementing this analysis for sinu-
soidally modulated inputs, allow accurate measurements of time constants
Sinusoidally modulated light inputs are now widely used in neurophysiological studies of both invertebrate and vertebrate visual systems (Cleland and Enroth-Cugell, 1966).

The visual systems of invertebrates have been analysed because they often exhibit functional characteristics in common with the more complex visual systems encountered in the vertebrates. Sensory neural functions of invertebrate compound eyes have therefore been widely studied to determine the basic principles which govern the processing of visual information. Limulus eye, a relatively simple visual organ, performs three distinct processing steps upon visual inputs. First, the transduction of a light input into an intracellular voltage (Fuortes, 1959; Rushton, 1959). This voltage in turn determines the frequency modulated spike signal which is sent to the brain (Fuortes, 1959).

Third, the firing of spikes determines the lateral inhibition which affects neighbouring eccentric cells (Hartline and Ratcliff, 1958). Knight et al (1970) measured the time-dependence of the individual steps involved in signal processing. Each of these steps acts as a linear transducer of its input. When the frequency responses of these steps are added, they predict that the overall transduction of light inputs to neural outputs in Limulus eye is linear.

The eye of Limulus and the human eye share a number of functional characteristics:

a). A great sensitivity to changes in light intensity as compared to a steady intensity level.

b). A graded response over a wide range of light intensities (to a factor of about seven log. units in intensity for Limulus).

c). An enhanced response to edges and contours (Knight et al, 1970).

As the relatively simple visual system of Limulus and the complex vertebrate systems exhibit common features, it is possible that a linear transduction mechanism is common to both.

Studies on the vertebrate retina have been mainly concerned with
ganglion cell activity (Cleland and Enroth-Cugell, 1966; Maffei et al, 1970; Hughes and Maffei, 1965; Schellart and Spekreijse, 1972). Hughes and Maffei, (1965) show, in cats, that the rate of ganglion cell discharges, in response to sinusoidal variations in light intensity, is determined by the input and is sinusoidal, especially at low frequencies. With increasing frequency the form of the response waveform remains essentially sinusoidal, even though distortion is apparent. This distortion is interpreted to be a consequence of complex non-linear neural or receptor mechanisms, which affect the retinal transfer characteristics. The presence of non-linearities, which affect the firing rate of the ganglion cells, are confirmed by Cleland and Enroth-Cugell, (1966). They studied four aspects of the stimulus-response relationship:

a) the mean discharge frequency;
b) the relative response amplitude;
c) the waveform of the response and
d) the phase shift.

From a total of thirty one cells they found only one cell which, at a constant input frequency, behaved linearly for small modulations of the input sine-wave.

This single cell exhibited the following four response characteristics, which relate to the Superposition Principle (Appendix) and define linearity:

1). The mean discharge frequency was independent of the modulation depth.
2). The stimulus-response phase shift relationship was independent of modulation depth.
3). The response amplitude was proportional to the depth of modulation.
4). The waveform of the response was almost sinusoidal.
These observations suggest that the transduction of light inputs to neural outputs cannot be fully described by a linear mechanism. Even though non-linear elements disrupt the characteristics of the cat ganglion cell responses measured in the above experiments, these results do not invalidate the use of steady-state analysis.

The experiments of Spekreijse, (1969) and Schellart and Spekreijse, (1972) show that the non-linearities do not affect the analysis of vertebrate systems, when small amplitude stimuli are used. These non-linearities are reflected in the nature of ganglion cell discharges, which allows them to be classified as "on", "off" and "on-off" types. Spekreijse, (1969) has shown that to a first approximation they result from a rectifying process which is assumed to be static. The distortion of ganglion cell responses therefore depends only on the amplitude and not the frequency parameters of the stimulus. The affect of non-linearities upon goldfish ganglion cell activity in response to both small amplitude Gaussian white noise and sinusoidally modulated light was studied by Schellart and Spekreijse, (1972). Their experiments show three characteristics of the ganglion cell responses:

1). The gain and phase data for sinusoidal inputs is predicted from the Gaussian noise analysis by two techniques. Firstly, a cross-correlation technique, which determines the impulse response, and secondly by Fourier transformation of the impulse response, to give the frequency response data for sinusoidal responses;

2). A linear relationship holds between response amplitude and the amplitude of an input sine-wave, and

3). The amplitude and phase characteristics in response to sine-wave stimuli are independent of stimulus modulation.

These observations show the similarity between sine-wave and noise data, and that the response characteristics are independent of the stimulus
amplitudes. This indicates that for small amplitude stimuli the non-linearities are invariant. As the Gaussian noise data predicts the sine-wave data, these observations show that steady-state analysis, when applied to small amplitude responses, describes retinal function.

The technique of steady-state analysis has been used to study the dependence of the dynamic characteristics of ganglion cell responses upon stimulus parameters such as intensity and spatial organisation. The mean intensity of a sine-wave stimulus affects the frequency response curves of both gain and phase. An increase in the mean of the sine-wave input results in the attenuation of the ganglion cell response at low frequencies (Schellart and Spekreijse, 1972). Because of this attenuation the gain characteristics of the response exhibit a peak at a certain frequency, which becomes more prominent with further increases in light intensity. The change in gain characteristics is accompanied by an alteration in the output-input phase relationship. Increasing mean intensity results in a decreased phase lag, especially at high frequencies.

Changes in the gain and phase characteristics of the ganglion cell responses also result from alterations in the spatial organisation of the stimulus. Schellart and Spekreijse (1972) show that, in goldfish retinas, an increase in the diameter of a stimulus spot, while maintaining constant flux per unit area, shifts the gain curve towards higher values on the frequency axis. These changes are accompanied by a decrease in the phase lag of the response. A second feature of spatial organisation is that it affects the response characteristics of the ganglion cells. Maffiì et al (1970) have shown that the frequency characteristics of the responses of ganglion cells in the cat retina are dependent upon the position of the stimulus spot in relation to the cell's receptive field. They found that, irrespective of cell type ("on" or "off"), the frequency characteristics of the responses evoked by central stimulation extend over a wider frequency range than those
evoked by peripheral stimulation. Based upon these observations, and histological evidence which shows that the dendritic spreads of ganglion cells appear to correspond to the centres of the receptive fields, not the entire fields (Dowling and Boycott, 1966), it is suggested that transmission of peripheral signals to the ganglia is mediated by interneurones unique to the peripheral receptive field.

The results from experiments designed to measure the dependence of ganglion cell responses upon various stimulus parameters suggest that a number of processes, for example, adaptation, feedback, and lateral interaction between neurones, play an important role in modifying the frequency characteristics of ganglion cells. To study such processes, which probably take place in the neural network distal to the ganglion cells, it is necessary to study the frequency characteristics of the individual neurones within the network. This approach has been used successfully by Toyoda (1974) to study the neural interactions in the carp retina. He measured the frequency characteristics of the receptor responses, the L-type S potentials arising from horizontal cells, the amacrine cell responses and the bipolar cell responses. Knowing the response characteristics of the individual cells in the neural network it is possible to assess the types of neural interactions which are involved in the transmission of information through the network.

At all mean intensities tested the amplitude characteristics of the receptor responses, as measured from the isolated mass receptor potential (distal PIII), have frequency responses which are flat over the low frequency range. This suggests that, in contrast to the visual cells of invertebrates, for example Limulus eye (Fuortes and Hodgkin, 1964; Knight et al, 1970), vertebrate receptors are not subject to an internal feedback control.

When the gain and phase characteristics of the horizontal cell responses are compared with those of the receptors, for inputs at a low standard mean intensity, there is little difference between the curves.
The horizontal cells however, show a marked low frequency attenuation at high light intensities. This attenuation can be explained by assuming a negative feedback loop exists between the horizontal cells and the receptors. Evidence exists to support this assumption, because when turtle horizontal cells are hyperpolarised by current it evokes depolarisation of the receptors (Baylor et al, 1971).

The frequency characteristics of bipolar cells in response to diffuse light, which stimulates their entire receptive fields, are similar to those of horizontal cells recorded at high mean intensities. These stimulus conditions result in an attenuation of the bipolar gain at low frequencies. However, when small light spots are used to stimulate the centre and surround responses independently, no low frequency attenuation is evident. This indicates that the frequency response characteristics, for whole field stimulation are a consequence of the antagonistic interactions between the centre and surround responses.

In comparison to the gain characteristics of bipolar cells, amacrine cell responses show a marked low frequency attenuation. Also, at low frequency, the output-input phase relationship exhibits phase lead. Toyoda considers that both of these phenomena are related to a process designated as neural adaptation, which involves both neural interactions and feedback mechanisms.

Studies upon the response characteristics of the individual neurones involved in the processing of visual information suggest that a number of complex neural interactions are involved in determining the ganglion cells output in response to light inputs. These studies indicate that both the lateral interactions between neurones and the modification of neural outputs by feedback perform an integral function in information processing.
The description of visual systems by analogy with the control theory of linear systems is useful in that it gives a coherent description of visual responses which incorporates, but is preferable to, descriptions of the individual aspects of the responses such as rates of rise, response amplitude and flicker fusion frequencies etc.

The use of linear control theory is restricted to those aspects of the response which are a linear function of the input. Its application to complex biological systems, the frog retina included, is hindered by their inherent non-linearities, e.g. adaptation. Although visual systems are inherently non-linear, a number of studies upon a variety of eyes, e.g. wolf spider eyes (Devoe, 1962; 1963); cat retinae (Hughes and Maffei, 1966); goldfish retinae (Schellart and Spekreijse, 1972) and the retinular cells of the desert locust and house cricket (Pinter, 1972); show that the responses of both vertebrate and invertebrate eyes can be reasonably calculated from a knowledge of the transfer characteristics within the linear range. Within this range the experimentally determined transfer function and the stimulus-response relationship it predicts for other inputs may be used as critical tests for the sufficiency of current hypotheses, concerning the mechanisms of visual information processing.

In the light of this knowledge, the mechanisms of information processing, within the frog retina, are investigated in this study by applying transient and steady-state analyses to the b-wave, off-effect and P_{III} component of the frog E.R.G. These analyses are performed to investigate the linear/non-linear characteristics of the frog retina and to establish if, by analogy with other visual systems, linear responses may be evoked by restricting inputs to a range of small amplitudes.

Having established linearity, the techniques of linear control
analysis are employed to investigate the relationship between both
the amplitude and temporal response characteristics of the E.R.G.
and the level of adaptation of the retina. A mechanism to explain
the effects of adaptation upon these parameters is proposed.

In addition to the E.R.G. study, maps of the spatial distribution
of sensitivity within the receptive fields of off-type ganglion cells
are constructed. The effects of this distribution upon the response
characteristics of off units, to stimulation with sinusoidally
modulated light focused at different positions within the receptive
fields, are described. A model to explain the position dependent
changes in these characteristics is outlined.
MATERIALS AND METHODS.
All of the experiments were performed upon retinal preparations from the excised eye of the frog (*R. temporaria*). Preparations made from the eye of this species were chosen for the following reasons:

a) The steady-state response characteristics of the E.R.G. and of the ganglion cell responses of the frog retina have been described by various authors.

b) The sclera is relatively rigid in comparison to that of other species, e.g., the rat (Brindley, 1958). The eyeball maintains its spherical shape when cut, allowing the cornea and lens to be removed without damage to the retina.

c) As the frog is cold blooded, retinal preparations from this species can be maintained in vitro without the need for precise temperature regulation or gassing with \( \text{O}_2/\text{CO}_2 \) mixture.

d) *R. temporaria* are easily available from suppliers at all times of the year, and can be maintained in good conditions with the minimum of attention.
The Preparation.

The frogs used were from a laboratory stock, which was kept in tanks of running water maintained at 10°C. Before the beginning of an experiment a frog was taken to the laboratory and pithed. In order not to expose the retina to bright light, which would cause substantial bleaching of the rhodopsin, the excision and dissection of the eye was carried out under dim red light. One eye was removed from the pithed frog by cutting the extrinsic eye muscles and the optic nerve and, depending upon the type of experiment to be performed, dissected to give one of two retinal preparations:

A). The exposed retinal preparation, and

Preparation A, which was used when recording the E.R.G. or the responses from single ganglion cells, was produced by cutting around the equator of the excised eye to remove the cornea, iris, ciliary body and lens. To allow oxygen to diffuse to the retina as much of the vitreous as possible was removed from the eye cup by absorption onto filter paper (Barlow, 1953a; Brindley, 1956). The opened eye cup was then placed into a small cup shaped depression drilled into a porous stone block (Brindley and Hamasaki, 1963). This block was transferred into a blackened perspex chamber with a small reservoir of Ringers solution in the bottom. The Ringers was absorbed into the block and its movement through the porous stone by capillary action kept the eye cup moist. The chamber was kept cool and moist by evaporation of Ringers solution from the surface of the block. Under the above conditions the preparation lasts for about an hour during which time the characteristics of the response remain fairly constant.

The light stimulus and recording micro-electrode were introduced to
the retina through a large hole in the lid of the chamber, the indifferent electrode entered through a hole in the side of the chamber, and then ran through a small hole in the stone block to make contact with the eye cup.

The $P_{III}$ component of the E.R.G. was obtained by the application of 50mM KCl Ringers solution to the receptor side of an isolated retina. The preparation was made from an excised eye with the cornea, etc., removed as described above. Following removal of the cornea, etc., the choroid and retina were separated from the sclera by running the points of a pair of blunt forceps along the junction between the sclera and the choroid (Hamasaki, 1962). Considerable care had to be taken to avoid damaging the retina, when breaking its main point of attachment around the optic nerve. The choroid and pigment epithelia were then floated free of the retina by placing a small drop of Ringers solution on the receptor side of the retina. Any remaining points of attachment, particularly around the optic nerve, were cut and the choroid plus pigment epithelia completely removed leaving the isolated retina intact. The preparation was placed receptor side upwards on a perspex block, which fitted into the darkened chamber.
Stimulation And Recording.

A). General Features.

Figure 1 illustrates the general features of the stimulating and recording apparatus, showing the basic units which were incorporated in the set up.

The stimulating apparatus was housed in a canopy of thick black cloth which prevented stray light from reaching the preparation. The preparation was stimulated by a spot of light which was variable in both intensity and diameter. This spot was produced by one of two optical stimulators, depending upon the type of experiment to be performed. The periodicity and duration of the stimulus was controlled by a Devices digitimer, which was used to switch an electromagnetic shutter. The stimulus waveform was monitored using a cadmium sulphide photo-electric cell, whose output was displayed upon a Tektronix 565 oscilloscope.

Recordings from a preparation were made using either 3M KCl or indium filled micro-electrodes. These recordings were amplified and the output of the amplifier was displayed upon the 565 oscilloscope and stored using either a Thermionic Instrumentation Recorder (series T30000), an S.I. Direct Recording Ultra-violet Oscillograph or by photograph from a slave oscilloscope. The frequency responses of the tape recorder and oscillograph were flat to 620 Hz and 6 KHz respectively, resulting in little, if any, attenuation of the recorded data.

B). Optical Stimulation.

Two forms of optical stimulation were used:

1). Sinusoidally modulated light around a constant mean intensity and
Figure 1. General features of the stimulating and recording set-up.
Optical Stimulator

Digitimer

Tape recorder

UV pen recorder

Oscilloscope (slave)

Preparation
(opened eye cup / isolated retina)

Oscilloscope (visual display)
2). Incremental and decremental step changes in intensity; additions or subtractions from a constant mean intensity.

1). The production of sinusoidally modulated light.

The optical stimulator used is illustrated in Figure 2. A 12 volt, 18 watt, straight tungsten filament bulb was used to produce a beam of constant intensity light. The filament of the bulb was always orientated in the vertical plane. In the event of a bulb burning out it could be replaced quickly by aligning the filament of a new bulb in the same plane.

The bulb was placed at the anterior focus of the lens \( L_1 \) (focal length 2mm). The output of the bulb therefore emerged from the lens as a parallel beam. This passed through an optical wedge \( W \), calibrated from 0 - 2.2 optical density units, in steps of 0.01 of a unit. The beam then passed through an infra red filter IF. This was necessary to produce a cold light, which did not heat the retinal surface.

The beam was polarised in the vertical plane by its passage through a polaroid \( P_1 \). The polarised light rays then impinged upon the surface of a half silvered mirror \( H_1 \), arranged at an angle of 45° to the path of the incident rays. This mirror splits the beam to produce two beams: one reflected through 90° at the mirror surface (beam \( P_2 \)) and the other transmitted through it (beam \( P_2 \)). The reflected and transmitted beams were equal in intensity, as measured by a cadmium sulphide photocell, so that each was equal to half of the intensity of the incident beam.

The reflected rays passed through the converging lens \( L_{II} \) (focal length 20mm) onto a plane mirror \( M_1 \). \( M_1 \) was orientated at an angle of 45° to the path of the incident rays. The rays were therefore turned through 90°, and brought to a focus on the rotating polaroid RP, which was at the focal length of lens \( L_{II} \). This polaroid was made to rotate by a D.C. motor. The rotational speed was varied by applying different
Figure 2. Apparatus for the production of sinusoidally modulated light.
voltages across the coils of the motor using a Variac. The maximum usable speed of the motor was six revolutions per second, above this speed the motor caused vibrations of the whole stimulator. The rotating polaroid acted as an analyser of the polarised incident beam. Therefore in consequence of its rotation the intensity of the transmitted light followed a sine wave. Due to symmetry of the rotating polaroid the frequency of the sine wave was double the rotation frequency. The intensity of the transmitted light formed the varying component of the sinusoidally modulated light stimulus.

The interaction of the rotating polaroid, with the polarised stimulus beam $P_1$ to produce the sinusoidally modulated stimulus, was monitored by following the intensity change of another polarised beam with a cadmium sulphide photocell. The position of the monitoring beam was such that its phase led the phase of the stimulus beam by $90^\circ$. As the phase of all responses was measured relative to the monitoring beam, in order to relate the phase to the stimulus $90^\circ$ had to be subtracted from all recorded phases.

The invariant component of the light stimulus came from the light beam $P_2$ originating from the fraction of the initial beam transmitted through the half silvered mirror $H_{S_1}$. This beam was made to converge by lens $L_{III}$ (focal length 20cm) to a focus on the equating polaroid $P_{II}$, following reflection through $90^\circ$ at the surface of the plane mirror $M_{II}$. Light emerging from $P_{II}$ fell upon the surface of a half silvered mirror $H_{S_2}$ at which point the two light beams $P_1$ and $P_2$ were combined.

The combined beam ($P_1$ and $P_2$) formed a divergent beam which impinged upon the lens $L_{IV}$ (focal length 10cm). The focal length of this lens was equal to each of the path lengths; $RP$ to $L_{IV}$ and $P_{II}$ to $L_{IV}$, therefore the divergent beam was made parallel by the lens. The parallel beam fell upon the iris $IR$. The light spots formed by each pathway, $P_1$ and $P_2$, were made the same size and superimposed upon each other, so that each one contributed the same percentage of light
transmitted through the iris. An electromagnetic shutter S placed in
the light path between lens $L_N$ and the iris allowed the light stimulus
to be switched on or off.

On calibrating the stimulator, the contribution made by the variable
pathway $P_1$ with the polaroids open and the constant intensity pathway
$P_2$ were measured. This was accomplished by placing a cadmium sulphide
photocell (see Foot note) in the combined pathway, $P_1 + P_2$, where it
emerged through the iris. Each pathway was then blocked in turn and
the resultant intensity measured by monitoring the output of the photo-
cell on an oscilloscope. Slight variations between the intensities of
each pathway were corrected by the addition of neutral density filters
to the appropriate pathway. The accuracy of the calibration depended
upon the correct alignment of the optical elements i.e. light rays had
to strike the surfaces of all mirrors at an angle of incidence of 45°
and the path lengths of $P_1$ and $P_2$ had to be equal.

Without filters the intensity of the light stimulus was composed of
50% unvarying light and 50% sinusoidally modulated light. Addition
of filters to either pathway altered the proportion of the varying to
invarying component, whilst appropriate alteration of the optical wedge
was used to control the mean intensity of the stimulus (Table 1).

Foot note. The maximum spectral sensitivity of a cadmium sulphide
photocell occurs for wavelengths within the range 500μm to 600μm. Zero
sensitivity occurs at about 800μm. The maximum operative range is, there-
fore, well below the maximum transmission wavelength of a pair of polar-
oids (axes parallel) which is greater than 700μm. The above relative
intensity measurements of pathways $P_1$ and $P_2$ would, therefore, be
inaccurate if their infra-red content were not accounted for. However,
infra-red (heat) filters are incorporated in both optical stimulators
(Figures 2 and 3) to reduce the infra-red (wavelengths in excess of
1250μm) in the stimulus beams to less than 20% of that emitted by the
light source.
Table 1.

The value of the filters required to produce attenuation of pathways $P_I$ and $P_{II}$ to give different modulation depths of the stimulus are shown, plus the appropriate alterations in the optical wedge required to maintain the mean intensity approximately constant.

<table>
<thead>
<tr>
<th>Modulation depth (%)</th>
<th>Value of neutral density filters (log. attenuation)</th>
<th>Mean intensity (lux)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_I$</td>
<td>$P_{II}$</td>
</tr>
<tr>
<td>99</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>91</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>66</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.6</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 3. Apparatus for the production of incremental/decremental step inputs.
2). The production of incremental and decremental intensity steps.

The optical stimulator used to produce incremental and decremental steps is illustrated in Figure 3. The design was based upon that shown in Figure 2 but some modifications were made in order to produce a range of step inputs; incremental between $+10\%$ to $+200\%$ of any given background intensity and decremental between $-10\%$ to $-100\%$. The modifications made were as follows:

a). The two light pathways $P_1$ and $P_2$ were powered by separate 12 volt, 18 watt bulbs.

b). An infra-red filter $F_{II}$ was placed in pathway $P_2$, so that the two pathways $P_1$ and $P_2$ were equivalent.

c). The shutter $S$ was used to switch pathway $P_2$ only.

Responses to incremental steps were elicited by adapting the retina to a background intensity produced along pathway $P_1$ and then exposing it transiently to light transmitted along pathway $P_2$. Responses to decremental steps, on the other hand, were produced by the addition of the intensities produced along both pathways, the shutter was then closed switching off pathway $P_2$.

The background intensity was set primarily by attenuating the maximum intensity of pathway $P_1$ with neutral density filters. Controlled attenuation of pathway $P_2$ produced a range of step inputs, plus or minus the background. A combination of neutral density filters and the optical wedge was used to attenuate pathway $P_2$. This allowed a greater degree of control than could be obtained using neutral density filters alone.

When recording the latency of responses to incremental, or decremental step inputs the shutter lagged behind the stimulus monitor. The lag was measured by placing a light activated switch in the light path. The time interval between the switching of the stimulus monitor and the activation of the light activated switch was then
measured. This difference equalled 30msecs, which had to be subtracted from all latency measurements.

The apparatus was calibrated to make each pathway equal at the beginning of an experiment, as described previously.

The light emerging through the iris, when using either optical stimulator, fell upon a mirror at an angle of 45° to the incident rays, and were reflected through 90° to fall upon a final microscope objective lens mounted above the preparation by a Narashige micromanipulator. The field of light reflected onto the lens was larger than the lens itself. A shield of black cardboard was therefore fitted around the lens mounting to prevent stray light from falling upon the retina. The light rays which were converged by the objective lens formed a spot of variable size on the retinal surface. The smallest spot diameter produced by the apparatus was 0.2mm, which was used for the study of receptive field organisation (Barlow, 1953b). The largest available size was 3mm. This was used to provide total field stimulation when recording the E.R.G. and its P_{III} component. The spot size was varied by moving the objective lens in the vertical plane, therefore altering the distance between the lens and the retinal surface. A spot diameter of 0.2mm was obtained when the distance between the lens and the retinal surface was equal to the conjugate focal length of the lens. The focus was conjugate because the stimulus beam falling upon the lens was divergent, causing the beam to be focused behind the focal point of the lens. To increase the spot diameter the distance between the lens and the retinal surface was made greater than the conjugate focal length. The diameter of the light spot produced by the objective lens at different distances from the retinal surface was found as follows:

The stimulus beam was focused onto a sheet of white paper, and using a calibrated microscope eye piece the diameter of the resultant
spot measured to be 0.2 mm. The conjugate focal length (F1) of the lens, defined as the distance of the lens from the paper measured on the vertical vernier scale of the micromanipulator, was measured. The distance between the lens and the paper was then increased until the spot diameter was measured to be 0.35 mm. The distance (D) of the lens from the paper was again measured on the vernier, and the increase in distance (D - F1) was calculated. This procedure was repeated for spot diameters of 0.5 mm, 0.75 mm, 1.0 mm and 3.0 mm.

As the light flux is inversely proportional to the area of the spot, when the diameter of the light spot increases the light flux upon the retinal surface will decrease unless compensating increases in the light intensity falling upon the objective lens are made. The compensations made when the spot diameter was increased from 0.2 mm to a new value, were calculated as shown below.

Calculation to determine the compensation necessary when the spot diameter is increased from 0.2 mm to 0.5 mm.

\[ \text{Flux} \propto \frac{1}{\pi r^2} \tag{1} \]

\[ \text{Flux} = \frac{K}{\pi r^2} \tag{2} \]

At a spot diameter of 0.2 mm \((r = 0.1 \text{ mm})\), the flux was assumed to equal 100%.

\[ \therefore \quad \text{From (2)} \]

\[ 100 = \frac{K}{\pi 0.1^2} \tag{3} \]
To find the new flux \( (x) \) at a spot diameter of 0.5mm \( (r_2 = 0.25\text{mm}) \),

\[
\therefore \quad \text{From (2)}
\]

\[
x = \frac{K}{\pi \cdot 0.25^2}
\] .......................... (4)

As \( K \) is a constant,

\[
\therefore \quad \text{From (3)}
\]

\[
K = 100(\pi \cdot 0.1^2)
\]

\[
\therefore \quad \text{From (4)}
\]

\[
K = x(\pi \cdot 0.25^2)
\]

\[
\therefore \quad 100(\pi \cdot 0.1^2) = x(\pi \cdot 0.25^2) \quad \text{Divide throughout by } \pi
\]

\[
\therefore \quad 100(0.1^2) = x(0.25^2)
\]

\[
\therefore \quad x = \frac{100(0.01)}{0.0625}
\]

\[
\therefore \quad x = 16\%
\]

From this calculation, when the spot diameter is increased from 0.2mm to 0.5mm the flux decreases to 16% of its original value. In order to compensate for this it was necessary to increase the total light intensity falling upon the objective lens, so that the flux upon the retinal surface was again 100%. This was achieved by removing the
appropriate value of neutral density filter from the light pathway
initial beam. The value of the neutral density filter was found from
the table of Light Transmission versus Density. In the above case, the
value of the filter which had to be removed was 0.8 optical density
units. As optical density units on a logarithmic scale, this represents
an increase in intensity of 6.4 times.

The intensities of the stimulus spots, used to adapt the retina to
different levels, were measured using an Avo light meter type 3,
calibrated between 0 to 500 lux (lm (m^2)^{-1}). As all of the spots were of
small diameters, which did not fill the light sensitive cell of the
meter, a comparator method was used to measure their intensities.

A sheet of white paper was placed upon the light sensitive cell and
a stimulus spot was focused on it. A light field, which was large
enough to fill the light sensitive cell, was then used to surround the
stimulus spot. The spot was viewed through a binocular microscope and
the intensity of the surround was varied until the spot just disappeared.
The sheet of white paper was removed and the intensity of the surround
was measured from the scale of the meter. This intensity was taken to
equal that of the stimulus spot. Ten measurements were made for each
intensity and the mean value calculated.

As the scale of the meter was calibrated logarithmically, low
intensity values could not be measured from it. These values were there-
fore calculated from a knowledge of the degree of attenuation required to
change a high stimulus intensity to a lower one.

The intensities used for adaptation of the retina, which include
constant background intensities and the mean intensities of sinusoidally
modulated light, were varied between 3 lux to 90 lux.
C). Recording.

Two types of recording electrodes were used depending upon the type of response being recorded. To record the E.R.G. and its \( \text{P}_{\text{III}} \) component, glass micro-electrodes filled with 3M KCl were used. The micro-electrodes used in all experiments were made from small bore glass tubing (Dade 50\( \mu \)l micro-pipettes) pulled on a Narashige micro-electrode puller. They were filled with heated 3M KCl solution by boiling under vacuum for 15 minutes (Nastuk, 1965) and left overnight in 3M KCl solution before use. Electrodes produced by this method showed a tip resistance of between 10-20 Meg-ohm, as measured in Ringer's solution.

To record action potentials from single ganglion cells, platinum tipped indium micro-electrodes were used. This type of electrode was produced using the method described by Nastuk, (1964). A small length of low melting point alloy (30\% indium and 70\% Wood's metal) was inserted into the shank of a micro-pipette (tip diameter 1 - 2\( \mu \)m). This was followed by a length of tinned copper wire approximately ten centimetres long. The neck of the electrode was then heated over a small electrical coil until the alloy was molten and pressure was then applied to the end of the tinned copper wire forcing the molten alloy through to the tip of the electrode. It was then tipped with platinum by electroplating in chloroplatinic acid. It was important to obtain a light covering of platinum on the tip. If a large deposit was formed the tip diameter was greatly enlarged, making isolation of single units difficult.

As the E.R.G. and its \( \text{P}_{\text{III}} \) component are D.C. responses and the action potentials recorded from ganglion cells are fast A.C. transients, it was necessary to employ a different recording and amplification system for the study of each type of response. To record the E.R.G.
and its PIII component a 3M KCl micro-electrode was mounted in a
Marashige micromanipulator. The electrode was advanced towards the
retinal surface by means of the micromanipulator until it just made
contact. The pressure exerted by the electrode tip upon the retinal
surface was then adjusted until the response was maximal. The output
of the electrode was coupled by a chlorided silver wire to the
recording side of a Burr-Brown Instrumentation amplifier (Type 3154/25).
The indifferent electrode was made of chlorided silver wire embedded
in the retinal mounting block. The amplifier was balanced before
the beginning of each experiment, and its capacity compensation
adjusted to give the shortest time constant for the rise and fall of a
square pulse test signal, without gross alteration of its waveform.

The output of the amplifier was fed to a Tektronix high gain
differential amplifier (Type 3A10) which was D.C. coupled. A 0.3KHz top
cut filter was interposed between the Burr-Brown amplifier and the
high gain amplifier. The frequency response of this amplification
set-up was flat to 30Hz and 6db down at 200Hz. Its output was
displayed on the Tektronix 565 oscilloscope and recorded for later
analysis.

To record action potentials from single ganglion cells, a platinum
tipped micro-electrode was wound onto the surface of the retina, using
a Marashige micromanipulator. The light spot was focused upon its tip
as described by Barlow (1953a) and the electrode then adjusted until
action potentials from a single cell were identified.

The output from the electrode was fed into one side of a Tektronix
(Type 2A61) high gain differential amplifier. The reference electrode
was a length of silver wire wound down onto the sclera using another
micromanipulator. This was coupled to the reference side of the
amplifier. The amplifier was used with a 600Hz bottom cut and a 60KHz
top cut filter in its input line. Under these conditions the frequency
response of the amplifier was tuned to give maximum output between 2KHz and 20KHz, therefore filtering out 50 cycle hum plus a considerable amount of fast noise, while amplifying the action potentials with a minimum of attenuation. The output of the amplifier was again displayed and stored as described.
Data Acquisition and Analysis.

Experiments were performed to examine the following E.R.G. response characteristics:

1) The degree of linearity of E.R.G. components, with special reference to the b-wave.

2) The gain or sensitivity of the E.R.G. components to incremental or decremental changes in intensity. Gain is defined as:

\[
\text{Gain} = \frac{\text{Output (µV)}}{\text{Changes in absolute intensity (Δ lux)}}
\]

3) Frequency dependent gain changes, as a function of the input frequency of sinusoidally modulated light.

The degree of linearity was determined by stimulating the retina with both sinusoidally modulated light and step inputs of light. Response amplitudes (µV's) are plotted against the input modulation depth for sinusoidal stimulation or the percentage change in background intensity for step inputs.

Gain was determined at a number of different background intensities by evoking responses to step inputs of light either in the positive direction; incremental steps, or the negative direction; decremental steps. The optical stimulator, illustrated in Figure 3, was used to perform these experiments. The responses to incremental steps in intensity were obtained by adapting the retina to a background intensity arising from pathway P_{1} of the stimulator. By opening the shutter, the light intensity of
pathway \( P_2 \) was superimposed upon the background. Decremental step responses were obtained by adapting the retina to the same background, but in this case its intensity was a sum of the intensities of pathways \( P_1 \) and \( P_2 \). By closing the shutter the intensity of pathway \( P_2 \) was subtracted from the background producing a decremental step. A range of incremental and decremental intensity changes were performed for each background intensity used. The response amplitudes of both the b-wave and off effect were measured in microvolts.

To measure frequency dependent gain changes, the stimulator in Figure 2 was used. The retina was stimulated with sinusoidally modulated light of constant modulation depth around a known mean intensity. The frequency of the stimulus was varied between 0.3 Hz and 12 Hz, in the form of a recording mirror (see next section), and the response amplitudes measured in microvolts. These amplitudes were expressed as values of dynamic gain in terms of the following equation:

\[
G = \frac{A_f}{A_s} \tag{1}
\]

\( A_f \) is the amplitude of the response at a stimulus frequency \( f \).
\( A_s \) is the amplitude of the response to a step input over the range minimum to maximum of the sinusoidal input modulation.

The usual equation for calculating \( G \) is:

\[
G = \frac{A_f}{A_{f(0)}} \tag{2}
\]

Where \( A_f \) is as above and \( A_{f(0)} \) is the reference amplitude recorded in response to an extremely low stimulus frequency. Both forms of equation, (1) and (2), give the same shape frequency curves when
gains are plotted against input frequency. They differ however in their relative placements of the curve in relation to the Y axis of the graph. This difference is of no consequence in this study because the position of the curves relative to the Y axis is determined by the gain of the mechanism responsible for the production of the E.R.G. components and their dependence upon the frequency of the input.

In addition to these experiments, the linearity and frequency dependent behaviour of "off" type ganglion cell responses were examined. The dependence of these characteristics upon the following two stimulus parameters were assessed:

1) The position of the stimulus spot within the receptive field of the ganglion cell.
2) Spatial summation.

The responses to known inputs were expressed as maximum instantaneous action potential frequencies, where instantaneous frequency is a measure of the frequency of an action potential with reference to the preceding potential; that is the frequency per second derived from the equation,

\[
\text{Spikes.s}^{-1} = \frac{1000 \text{ msec}}{\text{Interspike interval (msec)}}
\]

Maximum instantaneous frequencies were measured by eye from plots of instantaneous frequency versus either phase shift or milliseconds from stimulus onset depending upon the stimulus waveform employed (sinewaves in the former case, step inputs in the latter).
Criticisms.

A). The light stimulus.

1). In the production of sinusoidally modulated light, when the two polaroids, \( P_1 \) and \( P_{II} \), were crossed some light was still transmitted along pathway \( P_1 \). When two polaroids are crossed the light that is transmitted is in the blue region of the spectrum. No compensations were made for:

a) The contribution of the transmitted light to the total intensity produced by pathways \( P_1 + P_2 \).

b) The fact that the transmitted light was blue.

It was not thought necessary to make compensations to correct for points a) and b), because of the low level of transmission through the crossed polaroids which reduced the intensity transmitted along pathway \( P_1 \) to 0.0001% of the maximum value.

2). The size of the stimulus spot used for the stimulation of the receptive fields of ganglion cells was measured using a calibrated microscope eyepiece. Diffuse light around the spot was not measured with a comparator, as described by Barlow (1953b). The transition from a spot of constant high intensity to diffuse surround was estimated by eye. This transition was quite sharp so that the error in measuring spot size should be small.

B). Recording.

As previously mentioned the frequency responses of all recording and playback facilities were compatible with the responses which were recorded. The following criticisms can be made of the recording techniques and the recordings made:-
1). Lower noise recordings could have been obtained by the use of low resistance wick electrodes in preference to 3M KCl microelectrodes.

2). During the course of an experiment the E.R.G., especially the P_{III}, was prone to changes in response amplitude. Similarly, ganglion cell discharge patterns tend to change towards the end of an experiment. In order to compensate for any changes in response amplitude or discharge patterns, all observations were made in the form of a recording mirror i.e. when recording a frequency response the stimuli were presented in the following order:-

0 5, 10, 1, 8, 2, 4, 6, 4, 2, 8, 1, 10, 0, 5, Hz.

The mean of the two values was taken as the correct value, therefore compensating for any changes in the response during the period of the experiment. As can be seen from the order of presentation of the stimuli, this method averages the responses around the middle point, in this case 6 Hz. To avoid any skew in the results caused by always averaging around the same midpoint, the order of presentation of the stimuli was varied. This randomised the presentation and so minimised the effect of response decay on the overall collected results.

C). Analysis

When measuring the amplitude of the E.R.G. responses the mean of ten response amplitudes was measured for each observation. To determine the mean value for the maximum frequency of action potentials in response to a given input only three response maxima were used.
This number was considered to be a significantly large sample due to the consistency of the response under constant input conditions.

When measuring interspike intervals for the calculation of instantaneous frequencies, the distance between successive spikes was measured to the nearest 0.2 of a millimetre. The error in frequency measurement was therefore low for low frequencies but considerably greater at high frequencies where the interspike interval was small.

Estimations of the maxima for trains of spikes were made by eye from graphs of instantaneous frequency versus phase (or msec.). As this method is far from ideal and could tend to give subjective results, two general rules were obeyed in order to obtain a more objective assessment of the results:

(1) Maxima for trains of spikes were measured by giving most weight to the frequencies present in the greatest numbers, so that a train of spikes consisting of a large number of low frequency spikes was not given an inflated maximum due to the presence of a few spikes of high frequency.

(2) In a train of spikes following a low input frequency, when it was evident that the spike frequency was decaying exponentially from a maximum value, the decaying phase was not included in the estimate of the maximum.
RESULTS (I).
Evidence of linearity.

In response to a sinusoidal input the E.R.G. is non-sinusoidal (Figure 4). The positive and negative half cycles of the input evoke cornea positive responses (the b-wave and off effect respectively), suggesting that E.R.G. generation involves at least one non-linear process (Brindley and Westheimer, 1969). However, a plot of b-wave amplitude versus modulation depth (Figure 5a) shows that at a mean intensity of 90 lux the amplitude of the b-wave varies as a linear function of the input, for frequencies within the range 0.5Hz to 8.0Hz. Under the stimulus conditions employed, the non-linear element or elements involved in E.R.G. generation would seem to have little effect upon the amplitude characteristics of the b-wave. The phase difference between the peak of the b-wave and the maximum brightness of the stimulus sine-wave is plotted in Figure 5b. At a stimulus frequency of 0.5Hz, the response lags the input by 54°. The lag is nearly independent of the modulation depth of the input. At input frequencies of 2.5Hz, 4Hz, and 8Hz the phase lag is approximately constant at 112°, 154°, and 298° respectively. Although the lag increases with increasing stimulus frequency, the approximate independence between phase and input modulation depth holds. This independence is in agreement with the second condition of the Superposition Principle (see Appendix), and, as such, supports the view that the influence of any non-linear element upon the E.R.G. is reduced under the stimulus conditions employed.

The frequency dependent gain curves of the b-wave, in response to
Figure 4. Typical waveform of the frog E.R.G. in response to sinusoidal variations in input intensity, around a mean of 90 lux. Input frequency 1.2 Hz. (The numbers preceding each record denote the modulation depth of the input).
Figure 5. E-wave amplitude and phase as a function of sinusoidal modulation depth. Input frequencies: 0.5 Hz (●), 2.5 Hz (○), 4.2 Hz (Δ), and 8.0 Hz (▲). Mean intensity 90 lux.
sinusoidal inputs of two modulation depths around a mean intensity of 90 lux, are plotted on log/log co-ordinates in Figure 6a. The figure shows gain data recorded in response to modulation depths of 98% and 50% of the mean. The data are superimposed and can be fitted by a common curve (curve fitted by eye). This indicates that the gain of the system responsible for b-wave production is independent of the input modulation depth. Thus, under the stimulus conditions employed i.e. up to a modulation depth of 98%, the output per unit intensity change at a given mean intensity and frequency is constant irrespective of the absolute change in intensity.

The frequency dependent phase data (Figure 6b), corresponding to the gain data in Figure 6a, are obtained when the phase difference between the b-wave and the maximum brightness of the stimulus sine-wave is measured at different input frequencies. These data are also superimposed and may be fitted by a common curve. The phase relationship between the b-wave and the stimulus is, therefore, independent of the modulation depth of the stimulus, indicating that the temporal characteristics of the b-wave are determined by the mean intensity of the stimulus and not the absolute change in intensity around the mean.

To test the linearity of the b-wave amplitude over a wider range of input magnitudes than was possible using sinusoidally modulated light, incremental steps were used. The retina was adapted to a known background for three minutes to ensure that a constant level of adaptation had been reached. Incremental steps, of between 10% to 200% of the background, were then superimposed upon the background. Linearity was assessed under four different states of adaptation by using background intensities of:

90 lux, 27 lux, 7.5 lux, and 3 lux.

The amplitude of the b-wave in response to five different step inputs was measured at each background intensity and expressed
Figure 6. Frequency dependent gain and phase characteristics of the b-wave, in response to sinusoidal variations in light intensity of 98% (filled circles) and 50% (open circles) modulation. Mean intensity 90 lux.
in microvolts.

The results from one such experiment are presented in Figure 7 and Table 2. In the Figure, b-wave amplitude in microvolts is plotted against the percentage change in intensity for step inputs of 25, 50, 75, 100, and 200 percent superimposed upon background intensities of 90 lux etc.

The figure shows that when the retina is adapted to a background intensity of 3 lux, the b-wave amplitude increases linearly with step height throughout the whole range of inputs tested (25-200%). When the background is increased to 7.5 lux and above, the response amplitude is again linear for steps up to 100% of the background. For step inputs between 100% and 200%, however, the response begins to exhibit a non-linearity. At these background intensities the response of the b-wave to incremental steps can be divided into two distinct phases: first for inputs between 0 to 100% which show linear response characteristics and second between 100 to 200% which show non-linearity. As illustrated in the figure the change in gradient between the linear and non-linear portions of the amplitude versus input step curves increases with increasing background intensity. The system, therefore, becomes increasingly non-linear for step inputs above 100% as the background intensity is increased from 7.5 to 90 lux.

The increasing non-linearity of the system can be expressed numerically. If the b-wave amplitude (μV) is divided by the percentage increase in intensity, a value of microvolts per percentage change in background intensity (μV/ΔB) is obtained. For a linear system, this value would remain constant over the whole range of inputs used. However, non-linearities will result in a change in the value of μV/ΔB. When values of μV/ΔB for the responses evoked by step inputs between 0 to 100% are compared with the values obtained with step inputs in excess of 100%, an estimate of the increasing
Figure 7. Amplitude of the b-wave in response to step inputs, plotted as a function of the relative change in background intensity. Background intensities; 90 lux (filled triangles); 27 lux (filled squares); 7.5 lux (open triangles) and 3 lux (filled circles).
non-linearity of the system is obtained. The value of $\mu V/\Delta B$ for a 200% step progressively deviates from the linear value as background intensity is increased (Table 2). At a background of 3 lux the b-wave is linearly proportional to the input up to at least a 200% step, but when the background is increased to 90 lux the response to a 200% step is 3% less than linearity would predict.
TABLE 2.

Deviation from linear b-wave response characteristics, as background intensity is increased.

<table>
<thead>
<tr>
<th>Mean Intensity (lux)</th>
<th>( \mu V/\Delta B ) Linear Phase (A)</th>
<th>( \mu V/\Delta B ) 200% Step (B)</th>
<th>A/B</th>
<th>% Decrease in ( \mu V/\Delta B ) Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.18</td>
<td>1.18</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
<td>1.72</td>
<td>1.32</td>
<td>0.77</td>
<td>23</td>
</tr>
<tr>
<td>27.0</td>
<td>2.1</td>
<td>1.49</td>
<td>0.71</td>
<td>29</td>
</tr>
<tr>
<td>90.0</td>
<td>2.8</td>
<td>1.7</td>
<td>0.607</td>
<td>39</td>
</tr>
</tbody>
</table>
Transient Analysis.

The analysis of responses evoked by instantaneous changes in intensity such as step inputs can be used to determine the sensitivity changes, which result from changes in the level of adaptation of the retina. These variations are a result of changes in the gain of the mechanisms responsible for the transduction of light energy into sensory outputs (Rushton, 1962; Fuortes and Hodgkin, 1964).

To determine the gain of the mechanisms responsible for the propagation of the E.R.G. it is necessary to consider both the b-wave and the off effect. As these voltage transients occur as a result of different interactions between the components $P_I$, $P_{II}$, $P_{III}$, of the E.R.G. (Granit, 1933), each transient must be studied separately. Gain and its dependence upon the level of adaptation was measured by adapting the retina to each of six background intensities:

90 lux, 57 lux, 27 lux, 18 lux, 7.5 lux, and 3 lux.

An interval of three minutes was allowed for the retina to reach a steady state, i.e. a constant level of adaptation, and then incremental steps of known magnitude were superimposed upon the background (Brindley, 1956), or decremental steps subtracted from it.

Figure 8 illustrates the form of the b-wave in response to incremental steps of 98%, and 50% of the background, superimposed upon background intensities of 90 lux, 57 lux, 7.5 lux, and 3 lux. It can be seen from this figure that background intensity influences the shape of the b-wave. At low intensities, 3 lux and 7.5 lux, the peak of the response is rounded but as the background intensity is increased (from 7.5 lux up to a maximum of 90 lux) the peak of the responses becomes sharper. The size of the increment has little effect on the shape of the response, so that all responses evoked at a background of 3 lux appear slightly rounded, while all of those at 90 lux exhibit
Figure 8. B-wave responses evoked by incremental step inputs of 98% and 50% of the background. Background intensities (as by the horizontal column) 90 lux, 57 lux, 7.5 lux and 3 lux.

Note. Weber's law predicts that responses evoked by a given percentage input, at different background intensities, should be of the same amplitude. The amplitudes of the responses illustrated in the figure, however, vary because the ERG is only an approximate fit to Weber's law (see Figure 10).
sharper peaks.

Background intensity influences the latency of the initiation of the response and the time to peak. Latencies recorded at a background of 3 lux are consistently longer than those recorded at 90 lux.

The corresponding responses of the off effect to decremental steps subtracted from background intensities of:

- 90 lux, 57 lux, 7.5 lux, and 3 lux,

are illustrated in Figure 9. Both the response waveform and latency show the same dependence upon background intensity as is found with the b-wave, so that the response peak becomes rounded and its latency increases as the background is decreased.

To measure b-wave and off effect sensitivities the maximum potential difference, that is the potential at the peak (\( \mu V \max \)), was measured from step responses such as those illustrated in Figures 8 and 9. Division of this value by the absolute change in intensity used to evoke the response gives the sensitivity in units of microvolts per unit change in intensity, i.e.

\[
\text{Sensitivity} = \frac{\mu V \max}{\Delta \text{lux}}
\]

Sensitivity values, calculated from responses evoked by different absolute changes in intensity (incremental for the b-wave and decremental for the off effect) at a constant level of background illumination, are given in Tables 3A1 and 3A2. At a background of 3 lux (Table 3A1), b-wave and off effect sensitivities are fairly constant throughout the whole range of inputs tested, therefore confirming the linearity data of Figure 7. Consideration of the data obtained at a background of 90 lux, however, shows that b-wave sensitivity is only constant for inputs of 100% of the background and below. For an input of 200% the sensitivity drops considerably.
Figure 9. Off responses to decremental step inputs of 98% and 50% of the background. Background intensities (as indicated by the horizontal column) 90 lux, 57 lux, 7.5 lux and 3 lux. 

Note. The amplitudes of the off effects illustrated exhibit the same deviation from Weber's law as was observed for the b-wave.
TABLES $3A_1$ and $3A_2$

B-wave and off effect sensitivities (μV/lux) in response to different step inputs (Δ lux); the retina was adapted to background intensities of 3 lux and 90 lux.

### $3A_1$ - Background intensity 3 lux

<table>
<thead>
<tr>
<th>Step input (Δ lux)</th>
<th>% Change in intensity</th>
<th>μV/lux b-wave</th>
<th>off effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>200</td>
<td>42.1 ± 5.5</td>
<td>-</td>
</tr>
<tr>
<td>2.94</td>
<td>98</td>
<td>46.7 ± 3.0</td>
<td>39.0 ± 1.5</td>
</tr>
<tr>
<td>2.25</td>
<td>75</td>
<td>42.0 ± 2.0</td>
<td>44.0 ± 3.5</td>
</tr>
<tr>
<td>1.5</td>
<td>50</td>
<td>45.4 ± 2.5</td>
<td>36.5 ± 2.5</td>
</tr>
</tbody>
</table>

The data are given as means ± S.E. of at least 20 observations.

### $3A_2$ - Background intensity 90 lux

<table>
<thead>
<tr>
<th>Step input (Δ lux)</th>
<th>% Change in intensity</th>
<th>μV/lux b-wave</th>
<th>off effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>200</td>
<td>1.4 ± 0.33</td>
<td>-</td>
</tr>
<tr>
<td>88.2</td>
<td>98</td>
<td>2.2 ± 0.4</td>
<td>1.9 ± 0.15</td>
</tr>
<tr>
<td>67.5</td>
<td>75</td>
<td>2.5 ± 0.25</td>
<td>2.0 ± 0.22</td>
</tr>
<tr>
<td>45</td>
<td>50</td>
<td>2.6 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>
Sensitivity ($\mu$V/lux) of the b-wave and off effect as a function of background intensity: backgrounds 90, 57, 27, 18, 7.5, and 3 lux.

<table>
<thead>
<tr>
<th>Background Intensity (lux)</th>
<th>$\mu$V/lux b-wave</th>
<th>$\mu$V/lux off effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>2.5 ± 0.15</td>
<td>2.1 ± 0.15</td>
</tr>
<tr>
<td>57</td>
<td>4.25 ± 0.23</td>
<td>3.5 ± 0.20</td>
</tr>
<tr>
<td>27</td>
<td>8.0 ± 0.5</td>
<td>6.35 ± 0.30</td>
</tr>
<tr>
<td>18</td>
<td>9.4 ± 0.4</td>
<td>8.4 ± 0.30</td>
</tr>
<tr>
<td>7.5</td>
<td>22.6 ± 2.0</td>
<td>17.2 ± 1.25</td>
</tr>
<tr>
<td>30</td>
<td>46.5 ± 2.5</td>
<td>38.5 ± 1.55</td>
</tr>
</tbody>
</table>

Each sensitivity value is given as the mean ± S.E. of the pooled data (at least 30 observations) derived from step inputs within the linear response range (10%–98% of the background).
The effect of background intensity upon sensitivity is seen in Table 3B. This shows that an increase in background results in decreased sensitivity. A thirty fold increase in background from 3 lux to 90 lux decreases b-wave sensitivity by a factor of 18.6, and off effect sensitivity by 18.3. Under the stimulus conditions employed the changes in sensitivity are roughly equal. These changes are illustrated in Figure 10. Both sets of data are fitted by straight lines: slope -0.84 for the b-wave and -0.83 for the off effect (lines of best fit by the method of least squares). This indicates that b-wave and off effect sensitivities are related to background intensity by the following equations:

\[
\text{B-wave sensitivity} = A \cdot I^{-0.84}
\]

\[
\text{Off effect sensitivity} = A \cdot I^{-0.83}
\]

where \( A \) is a constant

\[ I \text{ is background intensity} \]

These relationships show that, within the range of intensities tested, Weber's Law which is given by the equation;

\[
\text{Sensitivity} = A \cdot I^{-1}
\]

is approximately valid for the frog E.R.C. This confirms Brindley's observations (Brindley, 1956) which were obtained within roughly the same intensity range.

Changes in the sensitivity of both the b-wave and the off effect are accompanied by changes in the time scales of the responses. This is illustrated in Figures 8 and 9 by the rounded nature of the responses at low background intensities (3 lux and 7.5 lux) as compared to the sharper peaked responses at higher backgrounds. The
Figure 10. Sensitivity of the b-wave (○) and off effect (□) as a function of background intensity. A line of best fit, by the method of least squares, is fitted to each set of data. Each data point is the mean ± S.E. of the pooled data in Table 3B.

For slopes of best fit lines see Text.
changes in time scale are quantified in Figure 11A. This shows that the time from the onset of the b-wave to its peak is approximately 200 msec when the retina is adapted to a background intensity of 3 lux. When the background is increased to 90 lux, the time is shortened to 150 msec. The time to peak of the response is therefore decreased by an increase in background intensity. This also applies to the rising phase of the off effect (Figure 11B).

The above changes in temporal characteristics reflect an intensity induced shortening in the time constants of the b-wave and the off effect, and, as such, indicate that the time constants are determined by background intensity. To verify this, incremental and decremental steps of 98%, 75%, and 50% of a constant background were used to evoke the b-wave and off effect respectively, and the time courses of the rising and falling phases of these responses were measured (Figure 12). In each case, the time course remains constant, irrespective of the input magnitude, indicating that the time constants of the response are determined by the background.
Figure 11. Effect of mean intensity upon the rise
time of the b-wave (A) and off effect
(B). Ordinate; amplitude at time t
after onset of the response, relative
to the peak amplitude of the response.
At each mean intensity employed; 90
lux(Δ), 27 lux(□), and 3 lux(○),
the step input was 98% of the mean.
Figure 12. Effect of step-input amplitude upon the rise time of the b-wave (A) and the off effect (B). Ordinate; amplitude at time \( t \) after onset of the response, relative to the peak amplitude of the response. The retina was adapted to a mean of 90 lux. Step inputs; 98% (□), 75% (Δ), and 50% (○).
Frequency Analysis

The typical E.R.G. response to sinusoidally modulated light stimuli, at mean intensities of 90 lux and 3 lux respectively, is illustrated in Figure 13. The waveform of the response, at any given frequency, is similar for the two levels of adaptation. At low frequencies the response is bi-phasic; one element of the response, the b-wave, corresponds to the positive half cycle of the sine-wave and the second, the off effect, to the negative half cycle. When the input frequency is increased the two responses move towards each other and begin to merge. At approximately 4.5 Hz the two responses are fully merged so the recorded response is mono-phasic and distinct on or off transients can not be distinguished. When the stimulus frequency increases the response amplitude is decreased.

Figure 14A shows Bode plots of the amplitude of the b-wave responses expressed as gain (as defined in Methods) from retinae adapted to three different background intensities. At 3 lux (open circles) the gain at 0.5 Hz is 32 μV lux⁻¹. Above this frequency, the frequency response curve falls off asymptotic to a slope of -0.5. As the frequency is increased the slopes of the asymptotes get steeper, until at between 8.0 Hz to 9.0 Hz the slope of the high frequency asymptote is -4. This is equivalent to a decrease in dynamic gain of 24 db per octave.

On increasing the mean intensity of the sinusoidally modulated light from 3 lux to 27 lux and finally to 90 lux, the form of the frequency response curve alters. At 27 lux (open triangles) the curve shows slight attenuation of the response at low frequencies. As the input frequency is increased from 0.5 Hz to 1.0 Hz the gain increases from 7.8 to 8.9 μV lux⁻¹. Above 1.0 Hz the gain begins to fall off asymptotic to a slope of -0.5. The gain characteristics there-
Figure 13. E.R.G. responses to sinusoidally modulated light of two mean intensities: 90 lux (vertical column A) and 3 lux (vertical column B), within the frequency range 0.3 Hz to 8 Hz. Stimulus frequency is shown preceding each recording. Amplitude calibration $G_1$ refers to 0.5 Hz and 0.94 Hz recordings at 90 lux; Calibration $G_2$ refers to all other recordings.
Figure 14. Bode plots of the frequency dependent gain and phase characteristics of the b-wave. Data recorded at a constant modulation depth (98%) around a mean intensity of 90 lux(□), 27 lux(△), and 3 lux(○). Each datum point is the mean (± S.E.) of at least 20 observations.
fore show a slight peak around 1·0Hz. As the input frequency increases, the slopes of the asymptotes get steeper until at between 8·0Hz and 9·0Hz the slope of the high frequency asymptote is -4.

At a mean intensity of 90 lux (open squares) the frequency response curve has a marked peak at 1·3Hz. The peak, which has a gain of 24 µV/ lux\(^{-1}\), results from low frequency attenuation of the response so that decreasing the input frequency from 1·3Hz, reduces the gain. At 0·5Hz the gain is down to 14 µV/ lux\(^{-1}\). For input frequencies above 1·3Hz the asymptotes to the decreasing gain curve get steeper as input frequency increases, resulting in a high frequency asymptote of slope -4. This slope is the same as that found when stimulating around mean intensities of 3 lux and 27 lux. Therefore, over the measurable frequency range of the b-wave, the high frequency asymptote does not vary from a slope of -4 when the retina is adapted to different levels. This observation strongly suggests that the mechanism responsible for the production of the b-wave is at least fourth order, but higher order dynamics cannot be excluded, because at all mean intensities the responses to stimulus frequencies in excess of 9·5Hz are lost in noise and cannot be measured.

As the level of adaptation is changed the frequency response curve is shifted on the x axis. Increasing light adaptation results in a wider frequency response, so that when the mean intensity of a sinusoidally modulated stimulus is increased from 3 lux to 90 lux the high frequency asymptote is shifted to the right. This shift is a consequence of a change in the time constants associated with the response, i.e. the effect of adaptation upon the time constants associated with the step response. The time constants associated with each frequency response curve were estimated by fitting first order asymptotes to each curve. As first order asymptotes have a
slope of -1, four of these combine to produce the high frequency asymptote of slope -4, measured for each level of adaptation. The break frequencies ($F_B$), i.e. the frequencies at which the slopes of the asymptotes to the curve change from 0 to -1, -1 to -2, -2 to -3, and -3 to -4, were then measured from the abscissa. From these values the time constants were calculated using the formula:

$$\text{(msec)} = \frac{10^3}{6.3 F_B}$$

These values are given in Table 4.

The measurements presented in Table 4, show that increasing the mean intensity of the stimulus results in a shortening of the time constants.

The previous data show that changes in the mean intensity effect both the dynamic gain and time constants of the b-wave. Another characteristic of the response, that is the phase relationship of the response to sinusoidal inputs, is also affected by mean intensity. In Figure 14B phase difference is plotted against frequency for the three mean intensities used. The phase difference between the response and the input increases as stimulus frequency is increased. At the three mean intensities 3 lux, 27 lux and 90 lux, the phase relationship changes from $-20^\circ$ to $-560^\circ$, $-15^\circ$ to $-540^\circ$, and $0^\circ$ to $-540^\circ$ respectively as the stimulus frequency is increased from 0.2 Hz to 9.0 Hz. The effect of increasing mean intensity is to decrease the phase lag of the response in respect to the input. This dependence of the phase relationship upon intensity is most evident for input frequencies between 1.0 Hz to 2.0 Hz, where for mean intensities of 3 lux and 90 lux the phase difference is approximately $70^\circ$. At both low (0.3 Hz) and high (9.0 Hz) frequencies this difference is reduced to about $15^\circ$ to $20^\circ$. 

Table 4.

The effect of mean stimulus intensity upon the time constants of the b-wave.

<table>
<thead>
<tr>
<th>Mean Intensity</th>
<th>Exponential Time Constants ( msec )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lux</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>27</td>
<td>64</td>
</tr>
<tr>
<td>90</td>
<td>58</td>
</tr>
</tbody>
</table>

Data given are measured from the break frequencies in the gain curves of Figure 14A.
The gain and phase characteristics of the off effect were measured over the frequency range in which the E.R.G. is bi-phasic. The measurements are limited to this range because it was not possible to isolate the off effect from the b-wave. Figure 15A shows the frequency response curves for the off effect recorded at mean intensities of 3 lux, 27 lux and 90 lux. This shows that the highest frequency at which gain determinations could be made, i.e. when the b-wave and off effect just merge, was approximately 2Hz when the mean intensity was 3 lux or 27 lux. Increasing the mean intensity to 90 lux, increased this frequency to around 5Hz. The off effect and b-wave therefore merge earlier at low mean intensities.

The three frequency response curves have the same general shape and show no low frequency attenuation. Increasing the mean intensity of the stimulus shifts the response curve to the right, indicating a shortening of the time constants. First order asymptotes fitted to the three curves result in high frequency asymptotes of slopes -2. This suggests that the frequency responses recorded at the different mean intensities all result from second order dynamics. The time constants relating to each curve were calculated as described previously, by recognizing the break frequencies in the asymptotes. The values are given in Table 5.

The phase data, corresponding to the frequency response curves of Figure 15A, are given in Figure 15B. This data is plotted relative to the minimum intensity of the sine-wave light input, which represents peak stimulation of an off sensitive component. The responses to low input frequencies of approximately 0.2Hz to 0.3Hz show a phase lag of about 90° for all three mean intensities tested. Increasing the stimulus frequency results in an increased phase lag. Within the range of input frequencies 0.2Hz to 2.0Hz the phase lag of the response increases from -100° to -220° and from
Figure 15. Bode plots of the frequency dependent gain and phase characteristics of the off effect. Data recorded at a constant modulation depth (98%) around mean intensities of 90 lux (filled squares), 27 lux (filled triangles) and 3 lux (filled circles). Each data point is the mean (± S.E.) of at least 20 observations.
Table 5.

The effect of mean stimulus intensity upon the time constants of the off-effect.

<table>
<thead>
<tr>
<th>Mean Intensity</th>
<th>Exponential Time Constants (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lux</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
</tr>
<tr>
<td>27</td>
<td>160</td>
</tr>
<tr>
<td>90</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>53</td>
</tr>
</tbody>
</table>

Data given are measured from the break frequencies in the gain curves in Figure 15A.
-90° to -180° for mean intensities of 3 lux and 27 lux respectively. When the retina is adapted to a mean intensity of 90 lux the phase lag increases from -90° to -220° for an increase in frequency from 0.2Hz to 5.0Hz.
Electroretinogram: \( P_{III} \) component.

Isolation of the \( P_{III} \) by application of 50mM KCl to the receptor side of the retina has detrimental effects, which result in a progressive decrease in \( P_{III} \) amplitude with time. It was, therefore, necessary to assess the functional state of the retina at intervals during an experiment. Figure 16 shows the normal E.R.G. response recorded prior to the addition of KCl to the receptor side of the retina, the isolated \( P_{III} \), and the E.R.G. after washing the retina with an excess of normal Ringers to reverse the effects of KCl. The waveform of the E.R.G. response after washing with an excess of normal Ringers is identical to that of the control response evoked before \( P_{III} \) isolation. This shows that the functional state of the retina has remained normal during KCl treatment, but as the amplitude of the response is reduced this indicates that KCl has had some effect on the retina.

The amplitude of the \( P_{III} \) component is progressively reduced during a sequence of KCl treatments. This is not too important, however, when measuring the gain characteristics of the response because gain is measured as a ratio, \( A_r/A_s \) (see Methods). This normalises the amplitudes recorded during an experiment to the amplitudes in response to step inputs presented immediately before and after the experiment.

Evidence of linearity

To test if the \( P_{III} \) component varies as a linear function of the input, sinusoidally modulated light around a mean intensity of 90 lux was used. Figure 17 shows the waveform of the \( P_{III} \) in response to this input. Modulation depths varied between 33\% to 99\% at input frequencies of 1.5Hz and 2.3Hz. At low input frequencies, e.g. 1.5Hz, the response is a replica of the input for modulation depths of 50\% and below. Above this value the response exhibits low frequency distortion. At higher frequencies, e.g. 2.3Hz and above, the waveform of the
Figure 16.  
1). B-wave and off effect recorded from a freshly isolated retina.
2). Effect of 50mM K+ Ringers on the same retina.
3). Addition of normal Ringers to the retina.
Figure 17. Typical waveform of the $P_{III}$ in response to sinusoidal variations in stimulus intensity around a mean intensity of 90 lux. Input frequencies of 1.5 Hz and 2.3 Hz. (The numbers preceding each record denote the modulation depth of the input).
response is free from distortion.

For input frequencies of 1, 2 and 4 Hz, the amplitude of the response (μ V) and phase relationship (degrees) were measured, and are illustrated in Figure 18A. This shows that the amplitude of the P_{III} in response to sinusoidally modulated light varies linearly with the modulation depth of the input. This linear relationship holds for all stimulus frequencies tested.

The phase data which corresponds to the above amplitude measurements is plotted in Figure 18B. At an input frequency of 1 Hz the phase shift is found to remain constant at 100° irrespective of the modulation depth of the input. When the input frequency is increased to 4 Hz the phase lag equals 220°. At a constant input frequency the phase relationship is therefore independent of modulation depth.

**Frequency Analysis.**

Figure 19 shows the effect of increasing input frequency upon the P_{III} component when the stimuli of two different mean intensities, 90 lux and 27 lux, are used. At both mean intensities, the responses show a low frequency distortion but this is less evident at the lowest mean intensity. With increasing frequency the responses resemble the input waveform more closely. The amplitude is, however, decreased and the phase lag between the input and the output increased.

Figure 20A shows Bode plots of the P_{III} amplitudes expressed as gain from retinas adapted to three mean intensities 3, 27 and 90 lux. The three curves show high frequency asymptotes with slopes of -4 and therefore represent fourth order dynamics. Increasing the mean intensity of the stimulus from 3 to 27 lux, and from 27 to 90 lux, shifts the frequency response curve progressively to the right,
Figure 18. $P_{III}$ amplitude and phase as a function of sinusoidal modulation depth. Mean intensity; 90 lux. Input frequencies: 1 Hz (○), 2 Hz (□), and 4 Hz (△).

Note. The large scatter in the data is probably due to the decay in $P_{III}$ amplitude, resulting from the detrimental effects of 50 mM KCl Ringers.
A

Amplitude (µV)

B

Phase (degrees)

Modulation depth (%)
Figure 19. The effect of increasing stimulus frequency upon the waveform, amplitude and phase of the P<sub>III</sub> recordings at two mean intensities: 90 lux and 27 lux. (Amplitude calibration C<sub>1</sub> refers to the 0.8Hz to 5.3Hz recordings at 90 lux; all other recordings made at calibration C<sub>2</sub>.)
indicating a change in the time constants. The time constants were measured from the break frequencies and their values are given in Table 6.

The values in Table 6 show a shortening of time constants with increased mean intensity, an observation previously seen with the b-wave and the off effect of the E.R.G.

The phase data corresponding to the frequency response curves is given in Figure 203. At the lowest mean intensity, the PIII lags the input by 120° at a frequency of 0.45 Hz. Increasing the stimulus frequency to 5 Hz, increases this lag to 400°. At the two higher mean intensities, 27 and 90 lux respectively, the phase lag varies between -90° to -360°, and -80° to -450° for an increase in frequency from 0.45 Hz to 10.0 Hz. An increase in mean intensity therefore affects the phase lag of the response. On increasing the mean from 3 to 90 lux, the phase lag at 0.45 Hz is reduced from 120° to -80°, a decrease of 40°. This shift in phase with a change of mean intensity is found throughout the frequency range tested. At high frequencies the shift is greater. At a mean intensity of 3 lux and a frequency of 5 Hz; the phase lags equals 400°, increasing the mean intensity to 90 lux reduces the lag to 280°. This represents an intensity induced phase shift of 120° as compared to 40° at low frequency.
Figure 20. Bode plots of the frequency dependent gain and phase characteristics of the 
P recorded at a modulation depth of 98%. Mean intensities; 90 lux (□), 27 lux (A), and 3 lux (○). Each datum point is the mean (± S.E.) of 16 observations.
Table 6.

The effect of mean stimulus intensity upon the time constants of the $P_{III}$.

<table>
<thead>
<tr>
<th>Mean Intensity</th>
<th>Exponential Time Constants (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lux</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>3</td>
<td>122 50 42 33</td>
</tr>
<tr>
<td>27</td>
<td>61 32 26 22</td>
</tr>
<tr>
<td>90</td>
<td>42 28 25 20</td>
</tr>
</tbody>
</table>

Data given are measured from the break frequencies in the gain curves in Figure 20A.
DISCUSSION (I).
The degree of linearity of the E.R.G. was assessed by focusing attention upon three aspects of its stimulus-response characteristics: the waveform of the response, response amplitude and phase shift at constant input frequency and the frequency dependent changes in gain and phase. If the frog retina was a linear input-output device then the E.R.G. in response to sinusoidal changes in light intensity would be a sine-wave of the same frequency as the input. However, as Figure 4 shows, the E.R.G. evoked by low frequency sinusoidal stimuli is non-sinusoidal, being diphasic and consisting of two cornea positive waves per input cycle. This indicates that, in common with E.R.G. generation in the baboon retina (Brindley and Westheimer, 1968) and the cat retina (Rodieck and Ford, 1969), generation of the frog E.R.G. involves at least one non-linear process. This agrees with the general observation that the transduction of light inputs to neural outputs, or the derivative field potentials, is non-linear (Brindley and Westheimer, 1968; Cleland and Enroth-Cugell, 1966; Hughes and Maffei, 1965; Rodieck and Ford, 1969).

Although the waveform indicates that a non-linear process is an integral component in E.R.G. generation, the stimulus-amplitude relationships of Figure 5a, which are derived from sinusoidal stimulation (mean intensities 3 lux to 90 lux), show that b-wave amplitude varies as a linear function of the input modulation depths up to 98%. This would suggest that, under the stimulus conditions employed, the mechanism of b-wave generation approximates to that of a linear system. This is supported by two further observations: the stimulus-phase relationships (Figure 5b) and the frequency dependent gain and phase (Figure 6), both of which are independent of the input amplitude at a constant frequency.

The above observations seem to be mutually exclusive, in that the
output of a non-linear system is essentially linear. To determine how such a situation arises the form of the non-linearity involved in E.R.G. generation must be defined.

Essentially there are two forms of non-linearity (Milsum, 1966):
(1) hard non-linearities which resist linearisation, and
(2) soft non-linearities, which can be linearised without affecting the qualitative behaviour of the system.

In biological systems hard non-linearities are not very abundant, although the threshold characteristics of neurones is one example. Soft non-linearities, on the other hand, are abundant. Included in this category are unidirectional rate sensitive elements, which in response to sinusoidal inputs give outputs resembling those of a half-wave rectifier.

Reconsideration of the b-wave, evoked by sinusoidal stimulation, reveals that it is evoked by the positive half-cycle of the input. This suggests that a unidirectional rate sensitive element and, as such, a soft non-linearity is involved in b-wave generation. This is supported by the linearity data, obtained in response to step inputs, which show that b-wave amplitude varies as a linear function of the input under restricted stimulus conditions, but becomes non-linear when the conditions are exceeded (Figure 7).

By analogy with rectifying processes previously studied (Spekreijse, 1969), the b-wave would seem to be generated by a non-linear element: - the effects of which may be reduced to a minimum, resulting in approximately linear response characteristics. As the b-wave is the algebraic sum of two potentials, the P_{II} and P_{III} (Granit, 1933), it is implicit in this conclusion that both components behave linearly under the stimulus conditions employed. To test the validity of this statement, the degree of linearity of the potassium chloride isolated P_{III} component was assessed using
sinusoidal stimulation around a mean intensity of 90 lux. Under these conditions $P_{III}$ waveform is approximately sinusoidal (Figure 17), suggesting linear behaviour. However, at modulation depths exceeding 50% slight low frequency distortion is evident but this is reduced as stimulus frequency is increased because the peak to peak variation in the non-linear element decreases as a consequence of the frequency dependent amplitude attenuation of non-linear R.C. networks (Pinter, 1966). As the distortion is slight, it suggests that there is little effect upon the linear characteristics of the $P_{III}$ itself. This is confirmed by both the amplitude and phase characteristics (Figure 18) which conform to the conditions of the Superposition Principle.

As a whole the linearity studies upon the b-wave, off-effect and $P_{III}$ component indicate that E.R.G. generation in the frog retina is influenced by one or more non-linear elements. These appear to be of a similar nature to those operative in the baboon and cat retinae. Thus, the non-linear response characteristics of the E.R.G.'s generated in all three retinae are similarly affected by stimulus conditions; restriction of the stimulus amplitude resulting in linearisation of the response characteristics.

When stimuli, in constant ratio to different background intensities, are presented to the retina sensitivity is decreased by an increase in background. This decrease occurs as a consequence of light adaptation. The results tabulated in Table 3B and illustrated in Figure 10 show that, within the intensity range employed, any change in background has similar quantitative effects upon the sensitivities of the b-wave and off-effect. Thus, when the relationship between background intensity and sensitivity is plotted on Log-Log axes a straight line of slope -0.84 fits the b-wave data and a straight line of slope -0.83 fits the off-effect data. From these data it follows that the changes in b-wave and
off-effect sensitivities are approximately described by Weber's Law (Brindley, 1956), in that sensitivity and background intensity are related to a first approximation by a reciprocal function, whereby:

\[
\text{Sensitivity} = A \frac{1}{I}
\]

where \( I \) = background intensity and \( A \) = a constant.

It is well established for invertebrate photoreceptors that a change in their sensitivity is accompanied by a change in the time scale of the response (Hartline and McDonald, 1947; Puortes and Hodgkin, 1964). The mammalian E.R.G. behaves similarly in that its temporal characteristics are influenced by the stimulus conditions employed, for example, an increase in the intensity of a stimulus used to evoke an E.R.G. from the dark adapted rat retina shortens the latency of the b-wave component. Extension of these observations to include b-wave latencies in response to stimuli superimposed upon steady backgrounds leads to the conclusion that both the latency of and the time to peak \( (t_{\text{max}}) \) of the b-wave are primarily determined (to within a few milliseconds) by stimulus intensity, rather than the state of adaptation of the retina or factors which determine b-wave amplitude (Cone and Platt, 1964).

In the frog, the \( t_{\text{max}} \) of either a b-wave or an off-effect, evoked by small amplitude step inputs superimposed upon a constant background intensity, is independent of the stimulus amplitude. If, however, a b-wave or an off-effect is evoked by stimuli of the same ratio of intensities: step input: background, but different background intensities, \( t_{\text{max}} \) is reduced by any increase in background. For the b-wave an increase from 3 lux to 90 lux reduces \( t_{\text{max}} \) from about 220 msec to 150 msec (Figure 11A). The same change reduces the off-effect \( t_{\text{max}} \) from about 450 msec to 200 msec (Figure 11B).
Under the stimulus conditions employed, background intensity rather than stimulus intensity would seem to determine the $t_{\text{max}}$ of the b-wave and off-effect. This implies that, within the linear response range, the temporal characteristics of the frog E.R.G. are determined by the state of adaptation and, as such, that adaptation determines the time constants of the E.R.G. components (b-wave and off-effect).

The effects of mean/background intensity upon response time constants may be analysed by plotting the frequency dependent gain and phase curves recorded at different mean intensities. It is easier to analyse these data, rather than transient response data because

1) A shift in the gain curve to the right on the frequency axis indicates a shortening of the response time constants.

2) The high frequency components of the response are better resolved.

3) The slopes of the frequency response curves allow identification of the components involved in the generation of the response, by analogy to known documented time functions (Milsum, 1969).

This form of analysis has been used to study invertebrate photoreceptor responses, and their dependence upon the level of adaptation (Dodge et al, 1968; Pinter, 1966; Knight et al, 1970). It has been found that light adaptation has a two fold effect upon the frequency dependent characteristics of these responses. First, any increase in mean stimulus level shifts the gain and phase curves to the right on the frequency axis, indicating that the time constants of the response have shortened. Secondly, at high mean intensities the responses are markedly attenuated at low frequencies, resulting in a resonant peak in the gain curve.
Interpretation of these changes is taken to indicate the existence of a time dependent gain control at the receptor level, which incorporates a feedback loop (Pinter, 1966).

The effects of light adaptation upon the potassium chloride isolated $P_{III}$ component are similar to those described above, however, low frequency attenuation is absent at all mean intensities tested (3 lux to 90 lux). These observations suggest that, although a time dependent gain control may be responsible for the intensity induced shortening of the $P_{III}$ time constants, feedback control is absent. Insight into the gain control mechanism may be obtained if the site of origin of the $P_{III}$ is known. This is difficult to determine because the $P_{III}$ is the sum of two potentials; one arising from the receptors (the distal $P_{III}$) and a second from the horizontal cells (the proximal $P_{III}$). However, the $P_{III}$ would seem to be primarily of receptor origin because its frequency dependent gain characteristics are similar to those of isolated cone responses and the vertebrate distal $P_{III}$ (Toyoda, 1974). This suggests that any change in $P_{III}$ gain or temporal resolution probably reflects adaptation at the receptor level.

At a mean intensity of 3 lux, the frequency dependent gain characteristics of the b-wave are similar to those of the $P_{III}$ in that low frequency attenuation is absent. Under these stimulus conditions, a simple transfer function may be used to describe the dynamic behaviour of the b-wave. To obtain this function the number of R.C. filters (Proctor and Hodgkin, 1964; Baylor et al., 1971) required to simulate the b-wave is found from the slope of the high frequency asymptote to the gain curve. The slope is -4, indicating that at least four stages of filter are required. From the values of the break frequencies, the time constants of
these filters are found to be 80, 40, 26 and 20 msec respectively.

A mathematical model of a system with the above time constants gives a good approximation of the frequency dependent gain characteristics recorded at 3 lux (Figure 21). However, the phase data predicted by such a model are in advance of the recorded stimulus-response phase relationship. With the addition of a time delay, such as might be expected for the photochemical processes underlying the response, the predicted phase relationship is retarded to give a good fit to the recorded data. The inclusion of a time delay has no effect upon the predicted gain because all signals are transmitted through a pure time delay without distortion. The model is represented by the following function, which is given in Laplace transform notation:

\[
H_s = \frac{e^{-0.045}}{(0.08s + 1)(0.04s + 1)(0.026s + 1)(0.02s + 1)}
\]

As the b-wave approximates to the output of a linear system, equation DI(1) should predict the behaviour of the system in response to any input. However, when used to predict the response to a step input the inadequacies of the simple transfer function are obvious (Figure 22). Although the rising phase of the experimental response is correctly predicted, the falling phase is not. The difference between the two responses suggests that an element must be included in the transfer function to account for the post peak repolarisation of the experimental response. A rectifying, non-linear element of the type already referred to would have the effect of producing post peak repolarisation. Such an element, which is represented by the transfer function:
Figure 21. (a). Synthesis of the "open loop"
(for restrictions in the use of this term see pgs. 87-88)
frequency dependent gain characteristics of the b-wave from Equ. (1).
Solid lines represent the gain curves of the four first order R.C.
elements: (time constants 80, 40, 26 and 20 msec). In cascade these elements produce the gain curve shown by the dashed line. Experimental gain curve, recorded at 3 lux, filled circles.

(b). Synthesis of the equivalent phase characteristics from Equ. (1).
Solid lines show the phase curves of the first order R.C. elements; the long dashed line illustrates the phase lag introduced by a 45 msec time delay. In cascade the above lags give the phase curve shown by the short dashed line. Experimental phase curve, recorded at 3 lux, filled circles.
Figure 22. Comparison of the h-wave step response, predicted by Eqn. (1) (filled circles), with the experimentally recorded step response (solid line). Ordinate; amplitude at time t after onset of stimulation expressed relative to the peak amplitude of the response.
where $A$ is a proportionality constant, will confer the property of unidirectional rate sensitivity upon the $b$-wave. The overall transfer function is then obtained:

$$
K_g = \frac{K_s}{T_s + 1} \quad \text{DI(2)}
$$

The time constants given in equations DI(1) and DI(3) only apply to responses recorded at a mean intensity of 3 lux. An increase in the mean to 90 lux results in a shortening of the response time constants (Figures 11 and 14) and attenuation of the responses evoked by low frequency stimulation (Figure 14). Shortening of the time constants in equation DI(3) could give an approximate description of the temporal characteristics of the $b$-wave at a mean of 90 lux, but would not predict the low frequency attenuation.

If the mean intensity induced changes in the frequency characteristics are to be explained in terms of a mathematical model, both the shortening of the time constants and low frequency attenuation must be predicted by the model. In the sphere of engineering science the appearance of the above response characteristics, upon increasing the mean input level to a system, is thought to indicate the existence of a feedback loop within the system. Similarly, where such changes in time-scale and gain are seen in invertebrate photoreceptors they are tentatively interpreted as showing the existence of a feedback loop at the receptor level (Puortes and Hodgkin, 1954; Pinter, 1966). Based upon these interpretations a feedback loop was closed around a transfer function

$$
E_S = \frac{e^{-0.045}}{(0.008s + 1)(0.004s + 1)(0.026s + 1)(0.02s + 1)} + \frac{K_s}{T_s + 1} \quad \text{DI(3)}
$$
similar to that of equation DI(1) (henceforth known as the 'open loop' function). In doing so, an attempt was made to predict the frequency dependent gain and phase data of the b-wave recorded at a mean intensity of 90 lux.

If the open loop function of a system is known, graphical methods are available by which the closed loop function may be synthesised. The simplest of these makes use of the Nyquist plot, in which the open loop gain and phase data are plotted on polar co-ordinates (Crodins, 1965) and then a vector analysis performed. Engineering criteria of stability (Evans, 1954) are employed to avoid unstable oscillations in the feedback loop. These dictate that the loop is closed with a maximum gain of x 1·4 at the frequency where the open loop gain is unity at a phase angle of -140°.

The closed loop response data for the b-wave were derived from the gain and phase data of Figure 23. These data are not a precise fit to either the predicted or the experimentally determined frequency response data for a mean of 3 lux, but they are within their limits. Thus, the frequency at which the open loop phase angle is -140° occurs at 1·5 Hz for the recorded data and 1·2 Hz for the predicted. The gain at 1·5 Hz is 0·5, indicating that a loop gain of 2 is required to produce the maximum closed loop magnification of x 1·4. When this gain is employed, the Nyquist plot (Figure 24) is obtained. From this plot, the closed loop gain at any frequency is calculated from the ratio of vector lengths (OF/O'F) while the corresponding phase is given by the difference A° - P°.

The predicted frequency dependent gain curve, as calculated by the above method, is plotted in Figure 25a, along with the experimentally determined values. This shows that closing a feed-
Figure 23. Comparison of the frequency dependent gain and phase data for the b-wave; derived from,

(1). The experimental data recorded at a mean intensity of 3 lux (o).
(2). Equation DI(1) (□).
(3). The data used to construct the Nyquist plot of Figure 24: (△).
Figure 24. Nyquist plot used to determine the closed loop gain and phase characteristics of the b-wave. (For explanation; see text).
back loop around the function depicted in Figure 23 gives a reasonable prediction of the temporal characteristics of the recorded responses, as evidenced by the following three points of agreement:

1) Both the predicted and experimental gain curves exhibit low frequency attenuation.
2) A resonant peak occurs in both gain curves at approximately the same frequency (within the error of the method), and
3) The high frequency ends of both gain curves may be superimposed.

Deviation of the predicted gain curve from the observed, particularly at the peak, is to be expected because the predicted response fails to account for the damping of oscillations at the system's natural frequency. In the physiological situation, it is probable that such oscillations are dampened by the saturation phenomena demonstrated in Figure 7.

The close agreement between temporal characteristics of the calculated closed loop function and the experimental data is confirmed and extended by the phase data, illustrated in Figure 25b. Thus, the predicted stimulus-response phase relationship is in good agreement with the experimental data.

Although the closed loop function gives a reasonable fit to the temporal characteristics of the b-wave and predicts low frequency attenuation it must be stated that the results do not provide real evidence that the b-wave is generated by a mechanism formally equivalent to a cascade of four B.C. filters, plus a feedback loop.

If the shortcomings of the above model are to be overcome, a physiological correlate to the model must be experimentally demonstrated. Thus, as the b-wave is generated across the bipolar
Figure 25. Frequency dependent gain and phase characteristics of the b-wave showing

(1). The open loop data (filled triangles) from which the Nyquist plot in Figure 24 was drawn.

(2). The closed loop data (filled squares) derived from the Nyquist plot.

(3). The experimental gain and phase curves (filled circles), recorded at a mean intensity of 90 lux; for comparison with (2) above.

Plot A -- Ordinate; gain expressed as

\[ \frac{A_f}{As} \]
cell layer (Hashimoto et al, 1961), it would seem appropriate to consider bipolar cell physiology.

Toyoda (1974) has made intracellular recordings from carp bipolar cells. Although these provide no evidence for feedback control of the cells' gain, they do reveal that the cells possess a complex centre/surround receptive field organisation. It is interesting to note that while the gain curves of the independently evoked centre and surround responses are flat over the low frequency range, simultaneous illumination of the centre and surround results in gain characteristics which exhibit low frequency attenuation. This arises because the centre and surround responses are approximately 180° out of phase at low frequencies, but are in phase at around 5 Hz. Thus, when both the centre and surround areas are illuminated simultaneously, the surround response augments the centre at an input frequency of 5 Hz but suppresses it at low frequencies.

In the absence of direct evidence for feedback control of bipolar cells, the intensity induced changes in b-wave response characteristics may be explained on a physiological basis if it is assumed that the centre/surround interactions are determined by the mean stimulus intensity. Within this scheme an increase in mean would enhance the centre/surround interactions and thus increase the degree of low frequency attenuation, resulting in a peak in the bipolar cell gain characteristics; a result which is similar to that found with the b-wave.
RESULTS (II).
Receptive Field Organisation Of Off Type Units.

The results presented in this section are from experiments designed to determine the effects of receptive field organisation upon the activity of the extrafoveal ganglion cells, which receive inputs from off type receptive fields. Before such a study can be made, single cell responses must first be isolated, and then identified as originating from these fields.

The isolation and identification of single off type units.

When the tip of an electrode makes contact with the surface of the retina, a large number of action potentials can be recorded at both the on and off of illumination. These responses consist of discharges arising from both ganglion cells and the axons which lie on the surface of the retina (Gemandt, 1948). When the electrode is advanced, so increasing its pressure on the surface of the retina, the number of action potentials recorded upon stimulation decreases until single unit recordings are obtained. Two types of single unit activity are found. First, and the most easily isolated, are polyphasic potentials. These are of characteristic waveform, illustrated in Figure 26(A), which is typical of the action potentials recorded from the nerve axons lying on the surface of the retina (Gemandt, 1948). The conclusion that the source is axonal is supported by the observation that these action potentials can be recorded when the stimulus is presented at a distance away from the electrode tip, which exceeds the known dimensions of the receptive fields for ganglion cells (Parlow, 1953a).

When the recording electrode is advanced, the polyphasic potentials are lost and a different form of action potential is then
Figure 26.  

a). Polyphasic action potentials, typical of those recorded from axons lying on the surface of the retina. Recorded at time scale $T_2$.  
b). Waveform of action potentials typical of those recorded from ganglion cells. Time scale; $T_2$.  
c). Action potentials of constant amplitude typical of a single ganglion, firing at low frequency. Time scale; $T_1$.  
d). Characteristic smooth decay in action potential amplitude, recorded from a ganglion firing at high frequency. Time scale; $T_1$.  
e). As (d) but recorded at time scale $T_2$. 


$T_1 \, 50 \, \text{msec}$

$T_2 \, 500 \, \text{msec}$
recorded, Figure 26(b). The waveform of this type of action potential is characteristic of the potentials recorded from retinal ganglion cells (Kuffler, 1951; Barlow, 1953a). As Figure 26(b) shows, initially the response evoked by light stimulation around the electrode tip consists of action potentials with different amplitudes, suggesting that the responses of a number of ganglion cells are being recorded simultaneously. Manipulation of the electrode reduces the number of action potentials constituting the response, until only those of constant amplitude remain (Figure 26(c)).

A characteristic feature of these potentials is the smooth decay in amplitude, which results from fatigue (Figure 26(d) and Figure 26(e)). This is typical of single cell recordings and rules out the possibility of the mass recording from several units firing in unison, which would result in a stepwise decay in amplitude. This evidence for single cell recording is supported by the observation that the action potentials, illustrated in Figure 26(c), (d) and (e), arise at sharp thresholds when the stimulus is presented on the electrode tip, or within an area of 1.0mm to 1.5mm in diameter surrounding it. On the interpretation of these observations, and on the confirmation by Rushton, (1949), Kuffler, (1951) and Barlow, (1953a), it is concluded that these action potentials originate from single ganglion cells situated within small receptive fields.

The receptive fields determine the activity of the ganglion cells in response to changes in the illumination of the retina. When small areas (0.1mm radius) of the retina are stimulated, the response characteristics of the individual ganglion cells are found to fall into one of three major classes: ON, ON/OFF and OFF (Hartline, 1940a; Barlow, 1953b). As this differentiation is a direct result of the cells receptive fields, the three classes of response also identify three receptive field types. On type fields possess
large excitatory receptive fields (ERFs), approximately 0.6mm in diameter, which are surrounded by inhibitory receptive fields (IRFs). Stimulation of the ERFs by light increments promotes maintained ganglion cell activity which ceases at the off. This activity is antagonised by simultaneous stimulation of the IRFs (Barlow, 1953b; Backstrom and Reuter, 1974). Receptive fields, which are classified as ON/OFF in the frog retina show no spatial organisation of the on and off components. Stimulation at any position within a frog ON/OFF type receptive field, with either incremental or decremental changes in intensity, therefore results in activity of the ganglion cell. This activity is phasic and lasts for a few milliseconds. OFF type fields possess large ERFs up to 1.4mm in diameter (Barlow, 1953b), which are only activated by decreases in illumination. This activity promotes a slow adapting ganglion cell discharge which can last for up to one second. In contrast to ON type fields, OFF type possess no IRFs (Barlow, 1953b; Backstrom and Reuter, 1974). As the response characteristics of each receptive field type are well defined, this allows for the immediate identification of the receptive field type responsible for any isolated ganglion cell response.
The influence of receptive field organisation upon the response characteristics of off type units.

The spatial organisation of off type receptive fields has been studied previously by Barlow (1955b). His measurements of the sensitivity at different points along the major axes of fields of this type suggest that at least two forms of spatial organisation exist: circular and elliptical.

The spatial distribution of sensitivity along the major axes of the receptive fields studied here are illustrated in Figure 27a. On the basis of this distribution, three classes of off type field are tentatively identified; designated classes 1, 2 and 3. In both classes 1 and 3, the spatial distribution of sensitivity is centred around high sensitivity plateaus (HSPs). In class 2 fields, however, the distribution is skewed so that the HSP is displaced towards one end of the field.

In addition to the type of experiment illustrated in Figure 27a, contour maps of equal sensitivity have been determined for some receptive fields and complete maps of sensitivity for others. In Figure 27b, contour maps of each class of field are illustrated. These extend the initial criteria used to differentiate between classes, by measuring both the boundaries of individual fields and the overall distribution of sensitivity within them. This allows a fuller description of each individual class than was possible using the previous technique.

Class 1 fields are circular and have diameters which vary between 1.4mm to 1.8mm. The sensitivity contours form concentric rings around the high sensitivity plateau, which has a diameter of between 0.3mm to 0.7mm. Class 2 fields, on the other hand, are approximately elliptical in shape with a long axis which varies from 1.2mm to 1.8mm, and a short axis of approximately 0.6mm. The plateau of high sensitivity is displaced along the long axis towards one end of the field, so that the spatial arrangement of the
Figure 27.  a). Sensitivity profiles through the receptive fields of off type ganglion cells. Ordinate; relative sensitivity --- reciprocal of threshold.

b). Contour maps of equal sensitivity, corresponding to the profiles in (a).
sensitivity contours is markedly skewed. Finally, class 3 fields are circular and show the same spatial arrangement as class 1 fields. They differ, however, as they possess much smaller diameters which vary from 0.9 mm to 1.2 mm. Of twenty fields studied, eleven fall into class 1, six into class 2 and three into class 3; representing incidences of 55%, 30% and 15% respectively.

The effects of spatial summation upon the thresholds of both class 1 and class 2 fields were investigated using stimulus spots with areas between 0.031 mm$^2$ and 0.44 mm$^2$. The changes in threshold which result from increased stimulus areas are illustrated in Figure 28. The threshold values recorded by stimulation of class 2 fields are consistently higher than those from class 1 fields, but have the same relationship to area in that a line drawn through each set of threshold values, measured for spot areas between 0.03 mm$^2$ and 0.19 mm$^2$, has a slope of -1. This implies that for stimulus areas up to 0.19 mm$^2$, i.e. within the limits of a receptive fields ESP, threshold is determined by a constant product of area and intensity ($A \times I = C$).

Figure 29 shows the gain and phase curves of a cell innervated by a class 1 receptive field, in response to stimulation of the ESP with sinusoidally modulated stimuli (spot areas 0.03 mm$^2$ and 0.19 mm$^2$). The power input ($A \times I = P$) to the ESP was held constant. Under these conditions, the gain curve with a 0.03 mm$^2$ is unaltered when the stimulus area is increased to 0.19 mm$^2$. The amplitude characteristics of the cell are, therefore, determined by the power and, as such, are independent of the stimulus area. The temporal characteristics of the cell are also determined by the power input: evidence the constant stimulus-response phase relationship recorded with stimulus spots of 0.03 mm$^2$ and 0.19 mm$^2$.

Figure 30 illustrates the variation in the number and frequency
Figure 28. Area-threshold relationship of ganglia innervated by class 1 (filled squares) and class 2 (filled circles) receptive fields. Thresholds are plotted as means (± S.E.) of at least 25 observations.
Figure 29. The amplitude (spikes sec\(^{-1}\)) and phase characteristics of a ganglion cell, innervated by a class 1 field, in response to stimulation of the field's HSP with sinusoidally modulated light. Two stimulus areas (0.03mm\(^2\) and 0.19mm\(^2\)) were used but the power input to the HSP was maintained constant.

* See Methods (page 39) for method used to determine frequency of spike output.
of action potentials recorded in response to sinusoidal stimulation at different positions along the major axis of a class 1 receptive field. Both the number and instantaneous frequency of the action potentials are maximal, when the stimulus spot is focused on the high sensitivity plateau of the receptive field (recordings labelled HSP in figure). Movement of the stimulus spot away from the HSP results in a decrease in both of these parameters. Comparison of the recordings labelled -0.2 and +0.2 shows that movement of the stimulus spot 0.2mm away from the HSP, to either the left or right, results in a similar change in the response characteristics.

The position dependent variations in the number and frequency of action potentials are accompanied by changes in the phase relationship between the response and the input. In the periphery the response lags the stimulus by approximately -180°. Movement of the stimulus spot towards the centre of the receptive field reduces the phase lag, until it is -45°, when the stimulus is focused on the HSP.

The changes in response characteristics with the position of the stimulus spot are also seen when a class 2 field is explored. The changes in both the number and frequency of action potentials, and their phase relationship to the input, are as would be predicted by the skewed nature of its sensitivity contours.

Having found that the response characteristics of a ganglion cell vary, when a supra-threshold stimulus is presented at different positions within its receptive field, the relationship between response amplitude (spikes sec⁻¹) and modulation depth of the stimulus was investigated. Figure 31 illustrates ganglion cell responses recorded during one such experiment, in which a sinusoidal stimulus of variable modulation depth was presented at three different positions within the receptive field of the cell. The
Changes in the response characteristics i.e. spike number, instantaneous frequency and phase, of a cell, innervated by a class 1 field, in consequence of stimulation at different positions within the field. The position of the stimulus, relative to the fields HSP is indicated by the numbers/letters preceding each trace. All stimulus parameters, except position, were held constant.
Figure 31. The effect of stimulus position upon the output of a ganglion cell in response to different modulation depths (within the range 98 - 20%) of a sinusoidally varying stimulus.

Vertical columns: traces recorded at a constant modulation depth, as indicated by the column headings.

Horizontal columns: recordings made at a constant position in the field, relative to the FSP. Letters/numbers indicate stimulus position.

Stimulus frequency (1Hz) and mean intensity (90 lux) were held constant throughout.
responses shown in Figure 31 (FSP) were evoked with the stimulus spot focused on the ESP. In this position the cell fires at all modulation depths tested i.e. 98% to 20%. If, however, the stimulus is moved towards the periphery of the field, the range of modulation depths over which the cell responds is reduced. Movement of the spot 0.2mm away from the ESP restricts the range to modulation depths between 33% to 98%, and a further movement of the spot decreases this range to between 50% to 98%.

In Figure 32 the response amplitudes (spikes sec⁻¹) at the three different receptive field positions are plotted against the percentage modulation of the stimulus. The response amplitude, recorded with the spot focused on the ESP, decreases from 115 spikes per second at 98% modulation to 27 spikes per second at 20%. The change in response amplitudes for modulation depths between 20% to 98% is fitted by a straight line which extrapolates to the origin. The responses therefore bear a linear relationship to the modulation depth of the input over the range of modulation depths 0 to 98%.

When the stimulus spot is moved to a position 0.2mm from the ESP (filled squares) the response amplitudes, at all modulation depths are reduced. The change in amplitude, over the range of modulation depths 33% to 100%, is again fitted by a straight line but in this case extrapolates to intercept the y axis at a negative value; -20 spikes per second. As the stimulus spot is moved further out towards the periphery, the value of this intercept becomes increasingly negative. This is a result of a further decrease in the overall response amplitudes and the reduction in the range of modulation depths over which the cell fires. In the extreme periphery (filled triangles) the negative value of the intercept decreases to -40 spikes per second.

The phase data corresponding to the above results are shown in
Amplitude (spikes sec$^{-1}$) and phase characteristics of a ganglion cell's output as a function of the modulation depth of a sinusoidally varying stimulus. The stimulus was focused at three positions within the cell's receptive field:

1. Upon the HSP (filled circles).
2. 0.2 mm from the HSP (filled squares).
3. In the extreme periphery (filled triangles).

* See Methods (page 39) for method used to determine frequency of spike output.
Figure 32b. At any position within the receptive field, the phase relationship between the input and the response remains constant irrespective of the modulation depth of the input. With the stimulus focused on the HSP the response lags the input by approximately $-90^\circ$. This lag increases to approximately $-180^\circ$ when the stimulus is moved to the extreme periphery, showing that the ganglion cell response exhibits a position dependent phase shift.

As the spatial organisation of a receptive field affects both the amplitude and phase of a ganglion cell's output in response to constant frequency stimulation, its affects upon the frequency response characteristics of ganglion cells were measured. Figure 33 illustrates the outputs of two ganglion cells (A and B) innervated by class 1 and class 2 receptive fields respectively, in response to sinusoidal stimulation at different frequencies. In each case, the stimulus spot was focused upon the high sensitivity plateau of the field.

Comparison of the response characteristics of each cell illustrates that two major differences exist:

1) The frequency response of cell A extends up to 8Hz whereas that of cell B only reaches 3.5Hz, and

2) in response to low frequencies cell A has a higher output than cell B, in terms of both the number of action potentials and their instantaneous frequencies.

Figure 34a shows Bode plots of the frequency response curves of both cells, in which the mean instantaneous action potential frequency is plotted against stimulus frequency on log/log coordinates. The frequency response curve of cell A (filled circles) has a marked peak at a stimulus frequency of approximately 1.0Hz. Above this frequency, the gain falls off asymptotic to a slope of $-\frac{3}{2}$. As the frequency is increased further, the asymptotes to the frequency
Figure 33. Ganglion cell outputs in response to sinusoidally modulated stimuli, within the frequency range 0.35 - 8Hz. Vertical column A; responses recorded from a cell innervated by a class 1 receptive field. Vertical column B; responses recorded from a cell innervated by a class 2 receptive field. Time calibration $T_2$ refers to responses recorded at an input frequency of 0.35Hz; calibration $T_1$ refers to all others.
Figure 34. Frequency dependent amplitude (spikes sec$^{-1}$)* and phase characteristics of the outputs of ganglion cells, innervated by class 1 (filled circles) and class 2 (filled squares) fields, in response to sinusoidally modulated stimuli (frequency range 0.3 - 8 Hz).

* See Methods(page 39) for method used to determine frequency of spike output.
response curve steepens, until the slope reaches -4 at an input frequency of 6Hz to 7Hz.

The frequency response curve of cell B (filled circles) peaked at approximately 0.6Hz. Increasing the stimulus frequency above this value results in a rapid decrease in gain, such that the high frequency asymptote; slope -6, occurs at a stimulus frequency of between 3.5Hz to 4Hz.

The phase data, corresponding to the Bode plots of Figure 34a, is illustrated in Figure 34b. The response of cell A is in phase with the stimulus at a frequency of 0.3Hz. Increasing the frequency results in increasing phase lag to reach a value of -300° at 8Hz. Cell B, however, shows a phase lag of -40° at 0.3Hz; increasing to -320° at 4Hz. As the rate of change of phase with stimulus frequency is greater for type B than for type A cells, the two sets of phase data diverge with increasing frequency. This divergence is in agreement with that observed for the gain curves, and illustrates a difference between the temporal characteristics of the two cells.

In the previous experiment, the frequency responses of cells A and B were recorded with the stimulus spot focused on the HSPs of their receptive fields. This determines the standard frequency responses, against which the effects of peripheral stimulation can be assessed.

In Figure 35, the output of a ganglion cell, innervated by a class 1 receptive field is shown. The responses, recorded with the stimulus spot focused on the HSP, are similar to those previously illustrated in Figure 33a, but movement of the stimulus spot towards the extreme periphery results in an alteration in the response characteristics. This movement has three effects:

1) At a given stimulus frequency, both the number and instantaneous frequency of the action potentials are reduced.
Figure 35. Effect of stimulus position, relative to the HSP of a class 1 field, upon the output of a ganglion cell in response to sinusoidally modulated light (frequency range 0.5 Hz - 8 Hz). Stimulus position is denoted by the horizontal headings; stimulus frequency by the numbers preceding each trace.
2). The phase lag, between stimulus and response, is increased.

3). The frequency characteristics of the cell are altered.

As a consequence of the effect upon the frequency response, the maximum frequency to which the cell will respond is reduced from 8Hz to 3Hz when the stimulus spot is moved 0.4mm from the FSP towards the periphery. A further reduction to 1.5Hz occurs when the stimulus is moved a further 0.2mm towards the periphery.

The effect of stimulus position upon the gain and phase characteristics of the cell are illustrated in Figure 36. These characteristics were determined with the stimulus spot focused at three different positions within the field. At the centre of the FSP, the gain curve is typical of that recorded from cell A (Figure 32a). When the stimulus spot is moved from the centre the gain curve is shifted to the left. Thus, a movement of 0.4mm from the centre shortens the bandwidth (Appendix) of the response from the initial value of 1.1Hz to 0.95Hz. A further reduction to 0.6Hz occurs when the stimulus is focused 0.6mm from the centre.

The phase data, corresponding to the above gain characteristics, is illustrated in Figure 36b. This shows that the temporal characteristics of the cells output are determined by a position dependent phase lag, which increases as the stimulus spot is moved from the FSP into the periphery of the receptive field.
Figure 36. Frequency dependent amplitude (spikes sec$^{-1}$) and phase characteristics of a ganglion cell output, in response to sinusoidally modulated light stimulus (frequency range 0.3Hz - 8Hz) focused at three different positions within a class 1 field. Field positions: HSP (filled circles), 0.4mm from the HSP (filled squares) and 0.6mm from the HSP (filled triangles).

* See Methods (page 39) for method used to determine frequency of spike output.

Each datum point is the mean of two observations.
Off-type receptive fields in the extrafoveal regions of the frog retina were found to fall into three major classes, designated 1, 2 and 3 according to the spatial distribution of sensitivity within the receptive fields, and the overall shape and size of the fields. In the primate retina there are significant differences in the sizes of the receptive field centres, which decrease as one moves towards the fovea (Hubel and Wiesel, 1960). Such a gradation was not observed in this study, although it is likely that, if present, it would not have been revealed by the random search method employed. However, the percentage distribution data show that receptive fields with large diameter high sensitivity plateaux (HSPs) are more evident in the extrafoveal retina than fields with small HSPs.

Information concerning the sizes and boundaries of HSPs is obtained from the area threshold curves of Figure 27. The sharp transition from the 45° line, which delineates the area of constant summation, to the horizontal line indicates that the HSPs of class 1 fields are circular and possess distinct boundaries (Rachstrom and Reuter, 1975). In contrast, the gradual transition seen for class 2 fields indicates that their HSPs are slightly elongated or elliptical.

The outlines of frog ganglion cell excitatory receptive fields (ERFs) are thought to correspond directly with the dendritic trees of the cells (Maturana et al, 1960). Similar conclusions apply to the ganglion cells of other species. Brown and Major (1966) have compared the dendritic trees of cat ganglion cells with the physiologically measured receptive fields (Rodieck and Stone, 1965) and conclude that the dendritic trees correspond to the centres of the receptive fields, not the entire receptive fields. In the frog dendritic trees with diameters which vary from 0.1 to 0.4 mm have been reported (Koch and Reuter, 1974). These diameters correspond
roughly with the HSP sizes of field classes 1, 2 and 3. It may be deduced from the comparative sizes of the HSPs and the ganglion cell dendritic trees that HSP responses are mediated by direct vertical pathways in the retina; receptor-bipolar-ganglion; without the need for lateral spread through interneurones: receptor-bipolar-interneurone-ganglion (Dowling and Boycott, 1966).

As the size of an entire receptive field is much larger than the dendritic spread of a single ganglion cell, peripheral receptive field responses must be mediated by interneurones. Evidence suggests that of the two types of interneurone in the retina: amacrine and horizontal cells, only amacrine cells are responsible for the lateral spread of peripheral responses (Dowling and Boycott, 1966). Thus transmission of signals from receptors in the periphery of a ganglion cell's receptive field will be along the neuronal pathway; receptor-bipolar-amacrine-ganglion cell.

Figure 37 shows one simple 'wiring diagram' for an off-type receptive field. This diagram may be used to explain some of the position dependent frequency response characteristics of off-type ganglion cells. In the scheme, which is similar to that for primate (Dowling and Boycott, 1966) and frog (Bachstrom and Reuter, 1975) retinal ganglion cells, the ganglion cell is in contact with bipolars only in the centre of the receptive field. Other bipolar cells in the field contact the ganglion via the amacrine cells.

Consideration of the vertical information-flow pathways of the HSP indicates that at any position within the HSP (consider receptors C₁ and C₂) receptor signals are transmitted to the ganglion cell along identical neuronal pathways. As the pathways include the same types and number of neurones, they will attenuate a given receptor signal to the same extent. The time constants of the responses evoked by stimulation at different positions
Figure 37 A "wiring diagram" of an Off-type receptive field based on the known anatomy of the retina. (For clarity only cones are shown in the scheme.)

Different areas of the receptive field are marked by the dotted lines:

H.S.P. ------ high sensitivity plateau.

P₁ ------ near periphery.

P₂ ------ far periphery.

For explanation see text.
within the boundaries of the HSP should then be equal.

Figure 36 shows that movement of a stimulus from the HSP to the periphery of an off-type receptive field lengthens the time constants of the ganglion cells responses. This observation poses a simple question: does the wiring diagram account for the change in time constants?

A knowledge of the dimensions (approximately 0.2mm or less) of commonly stained amacrine cells indicates that on moving from the HSP to the periphery an increasing number of amacrine cells are incorporated in the receptor to ganglion cell pathway (see Rodiech, 1973). Thus, in the 'wiring diagram,' receptors in area P1 are connected to the ganglion cell through a single interneurone; a receptor-bipolar-amacrine- ganglion pathway. In area P2 two interneurones are interposed between the receptors and the ganglion cell; a receptor-bipolar-amacrine-amacrine-ganglion pathway. As both neurones and interneurones act as low pass filters (Toyoda, 1974), which attenuate and phase retard receptor signals, peripheral responses from areas P1 and P2 should exhibit a greater degree of attenuation than responses evoked by stimulation of the HSP; the total attenuation and phase retardation being determined by the number of neurones/interneurones interposed between the receptors and the ganglion cell. Movement from the HSP to the periphery of the field depicted in the 'wiring diagram' should, therefore, result in a lengthening of the time constants of the ganglion cells responses; a result similar to that observed in the physiological situation (Figure 36). The scheme (Figure 37) may, therefore, be used to describe the position dependent changes in the time constants of off-type ganglion cells.

Many functional types of receptive field exist within the frog retina (Hartline, 1940; Barlow, 1953b; Maturana et al, 1960)
suggesting that information retrieval within the frog retina requires extensive preprocessing at the retinal level. It is evident in this observation that receptive fields capable of performing a large variety of functions must exist. It is not too surprising, therefore, to observe variations in the output characteristics of off-type ganglion cells (Figure 34) but if any significance is to be placed upon these variations, a role in information processing must be ascribed to them.

As Figure 34 shows, the HSP frequency response characteristics of cells innervated by class 1 and 2 fields differ considerably. The responses of each are tuned to respond maximally at different input frequencies, 1.0 Hz and 0.6 Hz respectively. Such a tuning phenomenon produces an overlapping of the frequency response characteristics of the two cells, so that the output of class 2 innervated cells is maximal when that of class 1 innervated cells is about half maximal. This overlapping may enhance the information transmitted along the optic nerve and thus reduce information loss at low frequencies.
APPENDIX.
PRINCIPLES OF SYSTEMS ANALYSIS.

Systems analysis allows mathematical modelling or representation of a system in terms of differential equations. Its basic assumptions are that any system will operate upon an input signal to produce an output. In consequence, any system can be represented as a "black-box" input-output device. If a mathematically defined input signal is used and the output monitored any divergence between the input and the output must be a consequence of the system's operation upon the input. The divergence between the input and the output can be used to characterise the system.

The existing theories of systems analysis are based upon linear differential equations, but it is well known that most biological systems, the retina included, contain non-linear elements. No analytical treatment is yet available for dealing with these systems but, when formulating a model of such a system, stimulus parameters can be usually be found which limit the system's behaviour to an approximate linear range (Devoe, 1963; 1964; Pinter, 1966; 1972; Schellart and Spekreijse, 1972). While it must be accepted that, in restricting a non-linear system to an approximate linear range of responses, a linear model cannot describe every aspect of a system's function, or the elements which constitute the system, it does provide a useful means of approach to and an understanding of the system's parameters. This leads to a useful insight into the dynamic interactions between the components of the system in providing the whole response.

To justify the description of a system's behaviour in terms of linear differential equations, the linearity of the system must be
experimentally proven. Thus, the output of the system, in response to the sine-wave input, must obey the following four conditions of the Superposition Principle:

1. The amplitude must be linearly proportional to the modulation depth of the input sine-wave at all input frequencies.
2. At a constant mean intensity and frequency, the phase relationship between the input and the output must remain constant for different input modulations.
3. At a constant mean intensity, the gain of the system should be independent of the modulation depth of the input.
4. The waveform should be sinusoidal.

If linearity is proven, two forms of analysis are available which can be used to characterise a system:

a) Transient analysis, and
b) Frequency/Steady-state analysis

A system is in steady-state when both the input and system variables are constant. If, however, the input is suddenly changed the output goes through a temporary adjustment or transient period before reaching a new steady-state value. Analysis of this transient, in response to either the unit impulse, the unit step or the unit ramp, characterises the dynamic properties of the system and thus provides information concerning the energy storage and dissipating elements of the system.

Figure 38 shows the transient response of a first order system, i.e. a system with a single time constant, in response to a step input. The behaviour of this system is described by the transfer function;
Figure 58. Step responses of two first order R.C. elements with different time constants. Ordinate: relative amplitude of response at time $t$ with respect to the time constant of the element.
The constant term $K$ determines the steady-state gain of the system, whereas the term $\frac{1}{Ts + 1}$ determines its temporal behaviour. The form of the step response is derived from the time derivative of the transfer function, and can be found from Laplace transform tables. Transformation of equation (1) yields the expression;

$$y(t) = \frac{Ke^{-t/T}}{T}$$

which shows that the first order step response is an exponential function of the time constant. The time constant therefore determines the time taken for the response to reach its steady-state value (compare curves of $T_1$ and $T_2$, Figure 38).

The behaviour of a second order system, i.e. one having two energy dissipating elements, depends upon the ratio of the two time constants (the damping ratio). As the second order transfer function is dependent upon the damping ratio it contains the possibility of resonance. The form of the transient responses of two second order systems, having different damping ratios are illustrated in Figure 39, showing the effect of damping upon the transient. Comparison of underdamped responses with the first order responses (Figure 38) shows that an underdamped second order system is characterised by oscillations in the transient, which are not present in the first order response. Overdamped responses, however, are non-oscillatory and, as such, may resemble first order transients. Under these
Figure 39. Effect of damping upon the second order transient response;
A). underdamped.
B). overdamped.
circumstances, it is difficult to discover the order of the system and steady-state analysis is best employed.

When the input to a system is a periodic function, usually sinusoidal, the input and system variables remain constant. This allows the parameters of the system to be identified by steady-state analysis. The output of a linear system to a sinusoidal input is also sinusoidal and of the same frequency as the input. The amplitude and phase of the output, however, depend upon the system parameters, $K$ and $T$ in equation (1). By altering the frequency of the input, at a constant amplitude, the changes in amplitude and phase of the output may be used to characterise these parameters, and the order of the system identified.

For a first order system, the gain $\left( \frac{K_{\text{output}}}{K_{\text{input}}} \right)$ decreases with frequency at 6 dB per octave, for frequencies in excess of the time constant. The gain therefore decreases by a factor of two per octave, which is equivalent to a line of slope $-1$, when both gain and phase are plotted on log./log. axes (Bode plots). Figure 40 shows Bode plots of two first order systems of different time constants. The frequency dependent gain curve is fitted by two asymptotes, one at low frequency with a slope of $-\frac{1}{2}$, and a second the high frequency asymptote with a slope of $-1$. The point of intersection of the two asymptotes; the break frequency ($F_b$), is used to determine the time constant ($T$) of the system from the expression;

$$F_b = \frac{1}{6.3T} \text{ sec}$$

where $T$ is measured in sec.

It can be seen that a change in the time constant shifts the frequency response of the system.

The figure illustrates the relationship between gain and phase. For a first order system, the phase lag increases from $0^\circ$ at zero...
Figure 40. Frequency dependent gain and phase characteristics of two first order R.C. elements with different time constants. Solid line; the asymptotes to the gain and phase curves, which are used in the synthesis of the frequency dependent characteristics of first order cascades. Dotted lines; the actual gain and phase curves.
Figure 41. The effects of damping ratio upon the frequency dependent gain and phase characteristics of a second order R.C. element.

A). underdamped.
B). overdamped.
frequency to $90^\circ$ as the frequency approaches infinity. At the time constant, the phase lag is $45^\circ$. If the time constant is changed the curve relating phase with frequency is shifted on the frequency axis.

Consideration of these relationships shows that for a constant input amplitude, the output amplitude decreases with frequency and, in consequence, the ability of the system to discriminate the input diminishes. Similarly as the phase lag increases, the time resolution of the system is less efficient.

The frequency response characteristics of a second order system are complicated by the existence of two time constants, which in combination determine the natural frequency, and in ratio damp its tendency to resonate. In Figure A1, the consequence of damping upon the tendency to resonate is illustrated. The high frequency response curves have slopes of $-2$, which distinguish them from first order systems.

The phase lag of a second order system increases from $0^\circ$ at zero frequency to $90^\circ$ at the natural frequency, and finally to $180^\circ$ as the frequency approaches infinity.

Analysis of complex physical systems, such as biological transducers, can be accomplished in terms of first or second order systems. Such high order systems usually comprise of an open chain or cascade of dynamic elements of the first or second order type or a combination of the two. At any frequency, each element contributes an appropriate gain and phase component to the overall response. To synthesise the frequency response of a cascade, the individual gains of each element are multiplied, while the phases are added. As gain and phase are related, the total gain contribution from each element within the cascade must be matched by the predicted phase shift.
ARDEN, G.B. and WEALE, R.A. (1954),
Nervous mechanisms and dark adaptation.
J. Physiol. 125, 417 - 426.

BACKSTROM, A.C. and REUTER, T. (1975),
Receptive field organisation of ganglion cells in the frog retina: Contributions from cones, green rods and red rods.

BARLOW, H.B. (1953a),
Action potentials from the frog's retina.
J. Physiol. 119, 58 - 68.

BARLOW, H.B. (1953b),
Summation and inhibition in the frog's retina.
J. Physiol. 119, 69 - 88.

BARLOW, H.B. (1964),
Dark adaptation: a new hypothesis.
Vision Res. 4, 47 - 57.

BARLOW, H.B. FITZHUGH, R. and KUFFLER, S.W. (1957),
Change of organisation in the receptive fields of the cat's retina during dark adaptation.
J. Physiol. 137, 338 - 354.
BAYLOR, D.A., FUORTES, M.C.F. and O'BRYAN, P.M. (1971),
Receptive fields of cones in the retina of the turtle.

BLAKEMORE, C.B. and RUSHTON, W.A.H. (1965),
The rod increment threshold during dark adaptation in
normal and rod monochromat.
J. Physiol. 181, 629 - 640.

BAUMANN, C.R. and SCHEIBNER, H. (1968),
The dark adaptation of single units in the isolated frog
retina following partial bleaching of rhodopsin.
Vision Res. 8, 1127 - 1138.

BRINDLEY, C.S. (1956),
The effect on the frog's electrinogram of varying the
amount of retina illuminated.
J. Physiol. 134, 353 - 359.

BRINDLEY, C.S. (1956),
Responses to illumination recorded by microelectrodes
from the frog's retina.
J. Physiol. 134, 360 - 384.

BRINDLEY, C.S. (1958),
The sources of slow electrical activity in the frog's
retina.
J. Physiol. 140, 247 - 261.
BRINDLEY, G.S. (1970),
Physiology of the retina and visual pathway.

BRINDLEY, G.S. and HAMASAKI, P.I. (1968),
The properties and nature of the R. membrane of the frog's eye.
J. Physiol. 167, 599 - 606.

BRINDLEY, G. and WESTHEIMER, H. (1968),
How deeply non-linear is the electroretinogram?
J. Physiol. 196, 78 - 79P.

BROWN, J.E. and MAJOR, D. (1966)
Cat retinal ganglion cell dendritic fields.
Exp. Neurol. 15, 70 - 78.

CLELAND, B. and ENROTH-CUGELL, C. (1966),
Cat retinal ganglion cell responses to changing light intensities: Sinusoidal modulation in the time domain.
Acta.physiol. scand. 68, 365 - 381.

CONE, R.A. and PLATT, J.P. (1964),
Rat electroretinogram: Evidence for separate processes governing b-wave latency and amplitude.
Science. 144, 1016 - 1019.

CRAIK, K.J.W. and VERNON, M.D. (1941),
The nature of dark adaptation.

Photochemical laws and visual phenomena.

DEVOE, R.D. (1962),
Linear superposition of retinal action potentials to predict electrical flicker responses from the eye of the wolf spider, Lycosa baltimoriana (Keyserling).
J. gen. Physiol. 46, 75-96.

DEVOE, R.D. (1963),
Linear relations between stimulus amplitudes and amplitudes of retinal potentials from the eye of the wolf spider.

DODGE, F.A., KNIGHT, B.W. and TOYODA, J. (1968),
Voltage noise in Limulus visual cells.

DOWLING, J.E. (1960),
Chemistry of visual adaptation in the rat.
Nature, Lond. 188, 114 - 118.

DOWLING, J.E. (1963),
Neural and photochemical mechanisms of visual adaptation in the rat.
J. gen. Physiol. 46, 1287 - 1301.

DOWLING, J.E. and BOYCOTT, B.B. (1966),
Organisation of the primate retina: Electron microscopy.
EVANS, W.R. (1954),
Control-system dynamics.

FUORTES, M.G.F. (1959),
Initiation of impulses in visual cells of Limulus.

Changes in time scale and sensitivity in the ommatidia of Limulus.
J. Physiol. 172, 239 - 263.

GERNANDT, B.E. (1948),
The form variations of the spike recorded by microelectrodes applied on to the mammalian retina.

GRANIT, R. (1933),
The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve.
J. Physiol. 77, 207 - 240.

GRODINS, F.S. (1963),
Control theory and biological systems.
Columbia U.P. New York.
HAMASAKI, D.I. (1962),

The effect of sodium ion concentration on the electroretinogram of the isolated retina of the frog.
J. Physiol. 167, 156 - 168.

HAMASAKI, D.I. (1964),

The electroretinogram after the application of various substances to the isolated retina.

HARTLINE, H.K. (1940),

The receptive fields of the optic nerve fibres.
Amer. J. Physiol. 130, 690 - 699.

HARTLINE, H.K. and McDONALD, P.R. (1947),

Light and dark adaptation of single photoreceptor elements in the eye of Limulus.

HARTLINE, H.K., WAGNER, H.C. and MacNICHOL, E.F. (1952),

The peripheral origin of nervous activity in the visual system.

HARTLINE, H.K. and RATCLIFF, F. (1957),

Spatial summation of inhibitory influences in the eye of Limulus and the mutual interaction of receptor units.
J. gen. Physiol. 41, 1049 -1066.
Localisation of the ERG by the aid of histological method

Receptive fields of the optic nerve fibres in the spider monkey.
J. Physiol. 154, 572 - 580.

Retinal ganglion cell responses to sinusoidal light stimulation.

A quantitative description of the dynamics of excitation and inhibition in the eye of Limulus.
J. gen. Physiol. 56, 421 - 437.

Unpublished.

Discharge patterns and functional organisation of mammalian retina.
J. Neurophysiol. 16, 37 - 68.
MAFFEI, L., CERVETTO, L. and FIORENTINI, A. (1970),
Transfer characteristics of excitation and inhibition
in cat retinal ganglion cells.
J. Neurophysiol. 33, 276 - 284.

MILSUM, J.H. (1966),
Biological control systems analysis.

MATURANA, H.R., LETTWIN, J.Y., McCULLOCH, W.S. and PITTS, W.H. (1960),
Anatomy and physiology of vision in the frog (Rana pipiens).
J. gen. Physiol. 43, 129 - 175.

NASTUK, W.L. (1963),
Physical techniques in biological research. Vol. VI.
Electrophysiological Methods, Part B.

NASTUK, W.L. (1964),
Physical techniques in biological research. Vol. V.
Electrophysiological Methods, Part A.

PINTER, R.B. (1966),
Sinusoidal and delta function responses of visual cells
of the Limulus eye.
J. gen. Physiol. 49, 565 - 593.
PINTER, P.P. (1972),
Frequency and time domain properties of the retinular cells of the desert locust (Schistocerca gregoria) and the house cricket (Acheta domesticus).

RODIECK, R.W. (1973),
The vertebrate retina: Principles of structure and function.
W.H. Freeman and Co. (San Francisco).

RODIECK, R.W. and STONE, J. (1965),
Analysis of receptive field of cat retinal ganglion cells.

The cat local electroretinogram to incremental stimuli.
Vision Res. 9, 1 - 24.

RUSHTON, W.A.H. (1949),
The structure responsible for action potential spikes in the cat's retina.
Nature. 164, 743 - 744.

RUSHTON, W.A.H. (1959),
Excitation pools in the frog's retina.
J. Physiol. 149, 327 - 345.
RUSHTON, W.A.H. (1962),
Visual adaptation.

RUSHTON, W.A.H. (1965c),
Bleached rhodopsin and visual adaptation.
J. Physiol. 181, 645 - 655.

RUSHTON, W.A.H. and WESTHEIMER, G. (1962),
The effect upon rod threshold of bleaching neighbouring rods.

SCHELLART, N.A.M. and SPEKREIJSE, H. (1972),
Retinal ganglion cell responses.

SPEKREIJSE, H. (1969),
Rectification in the goldfish retina. Analysis by sinusoidal and auxiliary stimulation.
Vision Res. 2, 1461 - 1472.

TOYODA, J. (1974),
Frequency characteristics of retinal neurones in the carp.

WALD, G. (1954),
On the mechanism of visual threshold and visual adaptation.
Science. 119, 887 - 892.
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K.A.P. GRATON R.Sc.

ABSTRACT

1) The effects of sine-wave and step inputs of light upon the frog (R. temporaria) electroretinogram were examined at different mean intensities. At all mean intensities tested (3 - 90 lux) the E.R.G. evoked by small amplitude stimuli is approximately linear, although inherent non-linearities are apparent. With larger amplitude stimuli the non-linearities become increasingly evident. At a mean intensity of 3 lux the b-wave evoked by small amplitude stimuli is approximately fitted by the equation for a linear filter with 4 RC elements, but the non-linearities have to be accounted for. An increase in mean intensity reduces b-wave sensitivity and shortens the time constants of the response. At a mean intensity of 90 lux the simple filter model does not describe the b-wave response characteristics. However, a reasonable fit to the experimental data may be obtained by closing feedback loop around the transfer function of the simple filter. It is tentatively suggested that a mechanism analogous to feedback may account for the shortening of the b-wave time constants caused by light adaptation.

2) The response characteristics of off-type ganglion cells were studied in response to sine-wave and step inputs of light. Upon the basis of receptive field size, shape and sensitivity distribu-
tion, three classes of receptive field are identified. Responses evoked by stimulation at different positions within a receptive field are compared and found to differ: peripherally evoked responses having longer time constants than FSP evoked responses. A model, based upon the known anatomy of the retina, is proposed to explain the position dependent changes in ganglion cell response time constants.