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A Neuropharmacological Evaluation of Visual Adaptation in the Isolated Frog Retina

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A NEUROPHARMACOLOGICAL EVALUATION OF VISUAL ADAPTATION IN THE ISOLATED FROG RETINA

by

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VITA

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In September, 1966, he married Veronica C. Coughlan. They have two sons, Michael and Brian.

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INTRODUCTION

The following is a basic review of the morphological and physiological characteristics of the vertebrate retina. It is an overview to aid the reader in his understanding of the experimental design and results obtained in this project against the background of the known morphology and physiology of the retina. Special reference will be made to the amphibian retina, the experimental animal used in the present study.

Interest in the structure and function of the retina has been demonstrated from antiquity, but the beginning of clear understanding of both awaited the development of microscopic techniques in the 17th century. The work of Treviranus in the 1830's was the first significant attempt to understand the function of the retina through the study of its minute structure, and gave powerful impetus to further investigation of this organ (Polyak, 1941). Most of the information we have today concerning the cell types in the vertebrate retina, and the form and distribution of their processes, has come from light microscopy of retinal tissue processed by the Golgi method (Dowling, 1970). The comparative studies of the vertebrate retina by S. Ramon y Cajal rest on this technique and remain today the classical description of retinal morphology on which all other studies

have been based. The studies of Polyak (1941), Dowling (1968) and Dowling and Boycott (1966) provide additional insight into retinal morphology and, together with Cajal's work, have permitted one to describe the following overall scheme of retinal structure.

The retina, broadly speaking, consists of five neural cell types: photoreceptor, horizontal, bipolar, amacrine and ganglion. Their cell bodies lie in three nuclear layers. Most synaptic contact between these cells takes place in two plexiform layers. The retina can be divided into six layers which are named in relation to their position to the center of the vitreous body. These layers are: (1) the outer segment layer - comprising the outer segments of the photoreceptors, (2) the outer nuclear layer - comprising the cell bodies of the photoreceptors, (3) the outer plexiform layer - in which synaptic contact between the photoreceptors, bipolar and horizontal cells takes place, (4) the inner nuclear layer - comprising the cell bodies of the bipolar, horizontal and amacrine cells, (5) the inner plexiform layer - in which synaptic contact is made between the bipolar, amacrine and ganglion cells, and (6) the ganglion cell layer - which consists of the cell bodies and axons of the retinal ganglion cells.

Spatial and temporal variations in light intensity

falling on the outer segments determine the visual information transmitted to the brain. This, however, is not a one to one transfer. Certain characteristics of these light patterns are abstracted and modified as a result of neuronal interaction within the retina. In general terms, there are two pathways allowing for information processing: (1) a straight-through pathway, composed of photoreceptors, bipolar cells and ganglion cells, and (2) a pathway allowing for the lateral spread of information, composed of horizontal and amacrine cells.

Photoreceptor cells

The photoreceptors are classified into rods and cones, which describe the morphological characteristics of the receptors. In the classical view the typical rod has a cylindrical inner and outer segment in which the diameters are similar, while cones have a conically formed outer segment and an inner segment with a diameter larger than the outer (Cohen, 1972). Schultze (1866) compared the retinas of diurnal and nocturnal animals and observed that the retinas of nocturnal animals were dominated by rods. His studies led him to propose that rods subserve scotopic vision (vision in dim light) and cones photopic vision (vision in bright light). This separation of photoreceptors into two functionally distinct types is termed the duplicity theory.

The frog retina contains approximately 58% rods and 42% cones; however, 98.3% of the total retinal pigment is found in the rods (Gordon and Hood, 1976). The frog photoreceptors can further be characterized as red rods, green rods, cones and twin cones (Cajal, 1972), each of which contains a visual pigment with a specific λ max (Liebman and Entine, 1968).

According to Draper's Law light must be absorbed to be detected. The light absorbing substances in the retina are the visual pigments and their bleaching by light was recognized as being fundamental to the visual process (Kuhne, 1878). Kuhne succeeded in extracting the pigment from the retina, demonstrating that bleaching resulted in the formation of various intermediates, and showing that visual pigment could regenerate. Fridericia and Holm (1925) discovered that Vitamin A was necessary for the normal regeneration of visual pigment, and Wald (1933, 1935) demonstrated the presence of Vitamin A, or retinol, in the retina. Wald and Brown (1950) found that retinal (the aldehyde of retinol) combines with the bleached fraction of visual pigment, called opsin, to form visual pigment. The visual pigment is currently considered to be a lipoprotein complex consisting of a single retinal moiety, a protein moiety (of approximately 235 amino acid residues), a single oligosaccharide moiety, and a phos-

pholipid moiety (Rodieck, 1973).

Hubbard and Wald (1952) observed that the chromophore, retinal, in its all trans-isomer form would not combine with opsin to regenerate visual pigment. 11-cis retinal was later demonstrated to be the important isomer in the generation of visual pigment, but when visual pigment is exposed to light the all-trans form of retinal is liberated (Wald and Brown, 1956). It was apparent that light causes a cis-trans isomerization of retinal. Hubbard (1958) suggested that the only action of light in the production of a visual signal is a cis-trans isomerization of the retinal group. Subsequent studies of the bleaching process have demonstrated a series of photoproducts leading from unbleached visual pigment to all-trans retinal and opsin. The following scheme, taken from Brindley (1970), gives the sequence of photoproducts as they have been identified to date:

Rhodopsin (500 nm)

↓

Prelumirhodopsin (543 nm)

↓

Lumirhodopsin (497 nm)

↓

Metarhodopsin I (478 nm)

↓

Metarhodopsin II (380 nm)

↓

Pararhodopsin (465 nm)

↓

N-Retinylidene-Opsin (440 nm)

↓

all-trans retinal and opsin (387 nm)

Photoreceptor outer segments contain an enzyme, retinal isomerase, which catalyzes the isomerization between all-trans and 11-cis retinal (Hubbard, 1956) so that regeneration of photopigment can take place within the photoreceptors. Kuhne (1878) observed that substantial regeneration occurred only if the sensory retina was in contact with the retinal epithelium and it is generally held that in the isolated, perfused retina regeneration of rod pigment does not occur. During light-adaptation all-trans retinal is taken up by the pigment epithelium and esterified. During dark-adaptation the ester is transported back to the rod outer segment (ROS) where it is isomerized to the 11-cis form. It has been suggested that the isolated retina has limited regenerative capability because the ROS isomerase is specific for retinyl ester, which under normal circumstances can only be provided by the retinal pigment epithelium (Bridges, 1976).

The isomerization of 11-cis retinal by light is the

initial event in a series of processes which leads to the production of a visual signal. The mechanism by which this photochemical event generates a potential change in the photoreceptor membrane (that is, the process of transduction) is not understood. Penn and Hagins (1972) demonstrated the presence of a dark photocurrent in retinal rods which is dependent on the inward flux of Na^+ ions. It is proposed that the absorption of light by the rod outer segments leads to the release of an internal transmitter which decreases Na^+ conductance, resulting in hyperpolarization of the photoreceptor (Hagins, 1972). It is further suggested that the internal transmitter is, in fact, Ca^{++}, which is liberated into the intracellular space of the ROS from intradisc space in rods and extracellular space in cones (Yoshikami and Hagins, 1973; Hagins and Yoshikami, 1974). Support for this hypothesis has come from 1) observations that changes in extracellular Ca^{++}, unlike Mg^{++}, K^+, Na^+ or Cl^-, have an action on photoreceptor membranes similar to that of light (Yoshikami and Hagins, 1973), 2) the ultrastructural localization of Ca^{++} within the discs of rod outer segments (Fishman et al., 1977) and 3) demonstration of the hyperpolarization of rods after intracellular injection of Ca^{++} (Brown et al., 1977).

Intracellular recordings from vertebrate photorecep-

tors have provided additional evidence for this overall scheme of receptor function. Neurons in the distal retina respond with slow potentials so that this approach is the only way to isolate the activity of these cells (Tomita, 1976). Tomita (1965) was the first to obtain intracellular recordings from photoreceptors, and to demonstrate that these cells are hyperpolarized by photic stimulation. Further studies showed that the hyperpolarizing response was always associated with an increase in the membrane resistance (Toyoda et al., 1969). Intracellular recordings from rods were first obtained in frogs by Toyoda et al. (1970). These cells also hyperpolarize to light to a maximum amplitude of 20 mV. The responses characteristically had a slow time-course and, with intense stimuli, the response greatly outlasted the flash, decaying very slowly. The spectral response of these cells peaks at about 500 nm. They were later identified as rods by intracellular staining. Intracellular recording from vertebrate photoreceptors has also been reported in the mudpuppy (Werblin and Dowling, 1969), gecko (Toyoda et al., 1969) and turtle (Baylor and Fuortes, 1970). All reports agree that the response to illumination is an hyperpolarization. This is in marked contrast to invertebrate photoreceptors which are depolarized by light (Tomita, 1976).

The mechanism by which a hyperpolarizing response in

the receptors is transmitted to second order neurons in the retina is not clear, especially since it is generally held that transmitter release is dependent on presynaptic depolarization. Trifonov (1968) observed a transient depolarization in horizontal cells (a second order neuron) following depolarization of receptor terminals. He felt this indicated that receptor terminals released transmitter when depolarized, which acted on horizontal cells. He proposed that transmitter substance is continuously released from the receptor terminals in the dark, and diminished or stopped when the receptors are hyperpolarized by light. The transmitter is thought to depolarize horizontal cells and the demonstration that these cells respond to photic stimulation by a hyperpolarization is in keeping with this hypothesis. Toyoda et al. (1969) have presented supportive evidence by demonstrating that the light-evoked hyperpolarization of horizontal cells is accompanied by an increase in input resistance. Furthermore, when synaptic transmission is blocked by perfusion of the retina with 1 mM $CoCl_2$, horizontal cells hyperpolarized and became non-responsive to light even though cones continued to respond normally (Kaneko and Shimazaki, 1975).

The mechanisms by which receptors communicate with more proximal neurons is still not clearly defined. An

alternative possibility — namely that hyperpolarization causes an increase in the rate of transmitter release — can not be ruled out, although its occurrence would be exceptional (Rodieck, 1973).

Photoreceptors make synaptic contact with second order neurons (horizontal and bipolar cells) in the outer plexiform layer. In general, two types of photoreceptor axonal endings can be recognized and correlated with rods and cones. Based on morphological differences, these endings are designated "spherules" in rods and "pedicles" in cones (Cohen, 1972). The synaptic contact between receptors and second order neurons is characterized by the presence of a dense bar or ribbon in the axonal ending lying next to the presynaptic membrane. Synaptic vesicles are found surrounding the ribbon in an orderly fashion. These synaptic ribbons, first described by Sjorstrand (1958), lie at the head of an invagination into the base of the receptor terminal. The post-synaptic elements of the receptor synapses are found within these invaginations, with three or more processes being present. It had been believed that the lateral processes within these invaginations represented horizontal cells while the central elements emanated from bipolars (Stell, 1965), but it has recently been demonstrated that this scheme does not always hold (Stell, 1976). In addition to ribbon

synapses, conventional synaptic contacts are also seen in the outer plexiform layer. The pre-synaptic elements are always horizontal cells, while the post-synaptic elements are horizontal or bipolar cells (Dowling, 1970). Within the outer plexiform layer visual information seems to be carried laterally by horizontal cells and vertically by bipolar cells (Dubin, 1974). In the frog, the outer plexiform layer is relatively thin and appears to be characterized by a lack of complexity when compared with the inner plexiform layer (Dowling, 1968). However, this appearance may be due to the minute dimensions of the neuronal processes involved and, in fact, in the rabbit a complex set of retinal functions has been shown to be involved with the outer plexiform layer (Sjorstrand, 1976).

Bipolar cells

The second order neuron in the transfer of visual information to more central areas of the central nervous system (CNS) is the retinal bipolar cell. The bipolar cells have their cell bodies located in the inner nuclear layer and send processes into both the outer and inner plexiform layers. In the frog, Cajal (1972) described two types of bipolar cells: 1) large or outer bipolar cells and 2) thin or inner bipolar cells. He suggested that the larger bipolar cells are associated with rods and the smaller with

cones. This association of function with a particular morphology is not clear, however Stell (1972) reports at least five types of bipolars in the predominantly rod retina of Mustelus. Similarly, Kaneko (1970) could find no morphological difference between two electrophysiologically distinct bipolar types.

Like photoreceptors, the bipolar cells generate only slow, graded potentials in response to light. Werblin and Dowling (1969) studied these cells in Necturus because of their relatively large size in this species. They observed two types of bipolar cell responses to illumination: 1) a hyperpolarization and 2) a depolarization. Furthermore, they observed that the receptive field of a bipolar cell consists of a central zone with an antagonistic surround. If the surround is stimulated simultaneously, the central response is diminished. Illumination of the peripheral zone alone, however, did not lead to a polarization of the cell in the opposite direction. The dendritic spread of Necturus bipolar cells has been estimated to be 80 - 100 μm (Dowling and Werblin, 1969) which corresponds to the central zone of the receptive field (Werblin and Dowling, 1969). It has been suggested, therefore, that the central zone is due to a direct activation of bipolar cells by the photoreceptors, while the antagonistic surround is mediated by the

activity of horizontal cells (Werblin and Dowling, 1969; Werblin, 1970).

In the transfer of information from the first to second order neuron there can be, at the same time, a great convergence and divergence of connections between photoreceptors and bipolar cells. Each bipolar cell can receive input from numerous photoreceptors, while individual photoreceptors usually make synaptic contact with a number of bipolar cells. In phylogenetically more advanced species this complexity tends to be reduced. This is especially evident in some primates where a single "midget" bipolar cell is in direct contact with only one cone photoreceptor and only one ganglion cell, giving rise to a direct straight-through pathway (Dubin, 1974).

Horizontal cells

Horizontal cells are retinal neurons having their cell bodies in the distal third of the inner nuclear layer and which send processes laterally through the outer plexiform layer. Cajal (1972) described two types of horizontal cells in the frog retina: 1) the outer horizontal cells, having small cell bodies, lying distally, and long, thin branching dendrites and 2) inner horizontal cells, having large cell bodies and short dendrites. The difference in size and shape of horizontal cells among vertebrate species,

especially in various teleost varieties, is very great (Stell, 1972). Dowling et al. (1966) described two types of horizontal cells in rabbit and cat: 1) large cells with no apparent axon and a dendritic field of 300 - 500 μm, and 2)small cells usually having an axon and dendritic fields of 100 - 200 μm. In monkey retina, horizontal cells have been designated as rod or cone type (Boycott and Dowling, 1969), although later studies have shown that dendrites of both types contact only cones while axon terminals make contact with rods (Kolb, 1970). It has been estimated that one horizontal cell contacts 6 - 30 cones through dendritic processes, while its axon terminals synapse with 80 - 150 rods (Gallego and Sobrino, 1975). One class of horizontal cell has been described which is particularly interesting. It consists of a cell body with a radiating system of dendrites and a single long, thin axon-like process which ends in an enormous terminal arborization. It has been demonstrated in the cat that the cell body receives signals predominantly from cones, while the terminal arborization receives input from rods. The long axon-like process plays no significant role in conducting these signals from one end of the cell body to the other (Nelson et al., 1975). A similar class of horizontal cell has been described in the salamander (Lasansky and Vallerga, 1975).

Like photoreceptors and bipolar cells, horizontal cells respond to light with slow, graded potentials. The responses are hyperpolarizing and sustained, with very slow time-courses (Werblin and Dowling, 1969). Svaetichin (1953) described a response to light from regions of the inner nuclear layer of fish retina which he ascribed to cones. These responses were later named "S-potentials" and their cellular origin remained in doubt for a long time. Finally, Kaneko (1970), using intracellular marking techniques, demonstrated that S-potentials are, in fact, produced by horizontal cells. S-potentials can be divided into two types: 1) L-potentials, or luminosity units, which hyperpolarize to all visual stimuli and 2) C-potentials, or chromaticity units, which hyperpolarize or depolarize depending on the wave length of the stimulus (MacNichol and Svaetichin, 1958). The receptive field of the S-potential is characteristically very large. Tomita et al. (1958) reported that these potentials could be influenced by retinal stimulation several mm from the recording electrodes.

The main pathway for lateral interaction in the outer plexiform layer is via the horizontal cells. These cells may be involved in the receptive field organization of the bipolars and in the adjustment of the dynamic range of these cells (Rodieck, 1973). Furthermore, it has been demonstrated

that more proximal neurons are also affected by horizontal cell activity. In the catfish retina artificial depolarization of the horizontal cell gives rise to ganglion cell responses similar to that induced with a spot of light, while artificial hyperpolarization gives rise to a response similar to that evoked by an annulus (Naka, 1972). Hyperpolarization of horizontal cells by extrinsic current leads to a depolarization of nearby cones (Baylor et al., 1971). This suggests that a negative feedback circuit might exist between horizontal cells and cones (Fuortes, 1972).

The second synaptic region of the retina is the inner plexiform layer. In all vertebrate retinas it is considerably thicker than the outer plexiform layer (Dowling, 1970). It is composed primarily of a) bipolar cell axons, b) amacrine cell processes, c) ganglion cell dendrites and d) Muller cell processes. The bipolar cell axons end in terminals containing a synaptic ribbon, similar to that found in photoreceptor terminals. In the inner plexiform layer, synaptic ribbons are only found in bipolar cells (Dowling and Boycott, 1965). Such synapses appear to be a characteristic of the bipolar cell. There are, generally, two postsynaptic elements at the ribbon synapse. This arrangement has been termed a dyad (Dowling and Boycott, 1965). The postsynaptic elements are ganglion and/or amacrine cell

processes. In the frog, postsynaptic processes of most ribbon synapses are from amacrine cells (Dowling, 1968). This distinction may be related to the more complex receptive field organization found in this species compared with that of phylogenetically more advanced organisms. Comparative observations indicate that in retinas which are dominated by "simple" receptive field organization bipolar cells make numerous direct contacts with ganglion cells, while in more "complex" retinas fewer direct bipolar - ganglion cell contacts and more intervening amacrine cell synapses are found (Dowling, 1970).

The inner plexiform layer also contains numerous conventional synapses. The presynaptic element is always an amacrine cell while the postsynaptic element may be a bipolar cell terminal, a ganglion cell dendrite or another amacrine cell process. Two types of conventional synapses are unusual. One is termed a reciprocal synapse and is characterized by the postsynaptic amacrine process of a dyad being presynaptic to the bipolar terminal a short distance from the ribbon synapse. This arrangement suggests a local feedback interaction. The second type is the serial synapse in which an amacrine cell process, postsynaptic to one amacrine cell, is immediately the presynaptic element in a conventional synapse with a third amacrine cell. The dif-

fering degrees of complexity among species in the receptive field organization of ganglion cells is also related to the degree of complexity found in conventional synapses in the inner plexiform layer. For example, Dubin (1970) found that less "advanced" species (with more complex receptive field organization) contained a higher ratio of conventional to ribbon synapses and a higher percentage of amacrine synapses in serial configuration.

In the inner plexiform layer, therefore, two different synaptic pathways to the ganglion cells may be postulated. One consists primarily of direct bipolar - ganglion cell connections. The second contains mainly bipolar terminals contacting amacrine cell processes, which interact among themselves and provide the primary input to ganglion cells.

Amacrine cells

Amacrine cells are, by definition, neurons without an axon. The cell body of these neurons lies in the proximal portion of the inner nuclear layer and cell processes are distributed throughout the inner plexiform layer. Cajal (1972) classified these cells, according to morphology, into two main types, diffuse and stratified. This classification is based on the manner in which the cell processes are distributed throughout the inner plexiform layer. In the frog, Cajal divided the stratified amacrine cells into five sub-

types according to the level in the inner plexiform layer in which the cell processes terminated. Boycott and Dowling (1969) followed the same scheme in general. This structural categorization, however, ought to be correlated with the functional characteristics of these cells.

Electrophysiological responses positively identified with amacrine cells were first recorded by Werblin and Dowling (1969). They observed that light-evoked responses consist typically of one or two spikes superimposed on a large, transient depolarizing potential that lasts for about 250 msec. Such responses are seen both at the onset and at the cessation of illumination. In frog, some amacrine cells produce large slow potentials with superimposed spikes, while others give rise to slow potential changes without spike activity (Matsumoto and Naka, 1972). The relationship between morphology and function in this class of retinal neurons is still not clear, however, and the association of spike discharges with neurons lacking axons is not fully understood. Observations in the catfish retina have led Chan and Naka (1976) to suggest that cells giving rise to sustained, slow potentials unaccompanied by spike discharges are, in fact, true amacrine cells; while cells associated with transient potential changes and spike activity may be a subclass of ganglion cells representing an alternative pathway of

retinal output. In any case, because of the complexity of observed responses the true nature of this class of retinal neurons may not be elucidated for some time.

Another class of neurons having cell bodies located among the amacrine cells which send processes to both the inner and outer plexiform layers, has been described in cats (Gallego, 1971), monkeys (Laties, 1972), dolphins (Dawson and Perez, 1973) and goldfish (Dowling and Ehinger, 1975). These cells have been called interplexiform cells and have been shown in goldfish and monkey to contain catecholamines (Dowling and Ehinger, 1975). In the goldfish, cell processes are pre- and postsynaptic to amacrine cells in the inner plexiform layer. In the outer plexiform layer these cells are always pre-synaptic to bipolar and horizontal cells. Interplexiform cells appear to provide a centrifugal pathway from the inner to the outer plexiform layers of the retina, and may be a general feature of all vertebrate retinas (Dowling et al., 1976).

Ganglion cells

The third order neuron, and final common pathway for visual information leaving the retina, is the ganglion cell. The perikaryon of this neuron lies in the ganglion cell layer and dendrites extend into the inner plexiform layer. Ganglion cell axons form the optic nerve and carry visual

information to the brain.

Cajal (1972) described two principal types of ganglion cells, diffuse and stratified, which he observed in all vertebrate classes. In the frog he described monostratified cells with dendritic terminals at a distinct level in the inner plexiform layer, bistratified cells, with dendritic terminals at two levels of the inner plexiform layer, and diffuse cells with ramifying processes and terminals throughout almost the entire plexiform layer. There are about 450,000 ganglion cells in each eye of the frog. Most of these cells (96%) have a small perikaryon, 7 - 10 μm in diameter, while the rest have cell bodies as large as 20 - 30 μm (Maturana et al., 1960). The number of these neurons appears to decrease from central to peripheral regions of the retina (Kalinina, 1976). Classification of ganglion cells in other species has been based on differences in cell perikarya (Polyak, 1941) or, like Cajal, on differences in the spread of cell processes throughout the inner plexiform layer (Boycott and Dowling, 1969).

The ganglion cell response to light stimulation is the generation of action potentials. These can be monitored by extracellular recording techniques. This has led to a wealth of information concerning the physiology of these cells and to classification schemes based on response pat-

terns to various forms of light stimulation. Hartline (1938) was one of the first to record responses from single ganglion cells in the vertebrate eye. He demonstrated that not all ganglion cells of the frog respond to light in the same manner. Some cells showed a burst of activity at the beginning of illumination followed by a steady discharge lasting throughout the stimulus duration. Other cells responded with a burst of impulses when the light was turned on and when the light was turned off, but no spike discharges during the steady illumination period. In a third type no impulses at all were seen during stimulation, but there was a large discharge of impulses when the light was turned off. Based on their response patterns these cells were classified as follows: 1) on-cells, 2) on-off cells and 3) off-cells. The region of the retina which must be illuminated in order to obtain a response in any given cell Hartline termed the receptive field. He noted that the receptive fields of most of the ganglion cells of the frog's retina were roughly circular, with a diameter of approximately 1 mm, and that the strongest responses were always obtained from the central portion.

A major advance in the concept of receptive fields and ganglion cell organization was made by Stephen Kuffler. Using small spots of light in the unopened cat's eye, he

found that the receptive field consists of a central area of low threshold and a peripheral region in which stimulation produces an opposing response pattern (Kuffler, 1953). Thus, if the central region produced an on-response, the surrounding region produced an off-response, and vice-versa. This center - surround organization explains why the discharge pattern of individual ganglion cells is not fixed but depends on certain factors such as background illumination, extent and location of retinal area stimulated, etc. This functional organization has been found in other species and may be a general feature of the vertebrate visual system.

Variations in photic stimulation have revealed a wide variety of functional units within the ganglion cell population. Using sinusoidal gratings over the receptive field, Enroth-Cugell and Robson (1966) described x- and y-cells in the cat: the former not responding to patterns of illumination if the net luminous flux for the receptive field remained constant, while the latter always responded to the introduction of a patterned stimulus.

It was further demonstrated that x-cells respond with sustained activity in the presence of an on-going effective stimulus, while y-cells only give transient responses (Cleland et al., 1971). Stone and Hoffman (1972) observed ganglion cells in the cat retina which were not character-

ized by a center - surround organization and which had much slower conduction velocities than x- or y-cells. They termed these cells w-units.

In the rabbit, Barlow and Hill (1963) demonstrated a class of ganglion cells with receptive fields organized to respond optimally to stimuli moving in a certain direction. Using small spots of light, they showed that these cells respond vigorously to movement in the preferred direction, but produced no response to movement in the null direction. Other classes of ganglion cells have been described which, in the final analysis, are dependent upon patterns of response to varying stimulus parameters. The relationship between functional organization and morphology has yet to be clearly established.

In the frog, the classification of ganglion cells has been most completely described by Maturana et al. (1960). They described five classes of ganglion cells based on the "operation" performed by each type. Class 1 - sustained edge detection: these cells do not respond to general changes of illumination; however, the sharp edge of an object moved through the receptive field produces a burst of activity. Class 2 - convex edge detection: these cells respond with a strong burst of activity to movement of a small object darker than the background exhibiting a sharp

edge. Class 3 - changing contrast detection: these units respond to the turning on and turning off of light with small bursts of 2 to 4 spikes. Class 4 - dimming detection: these cells give a prolonged response to the cessation of light. Class 5 - dark detection: these units are continuously active but their activity is inversely proportional to light intensity and increases to a maximum in darkness. All classes appear to be uniformly distributed across the retina. The complexity of ganglion cell receptive field organization in the frog is in keeping with a general trend toward more information processing occurring at the level of the retina in phylogenetically less advanced species. For example, complex retinas are described for frog and pigeon, mixed retinas are seen in rabbit and ground squirrel and simple retinas are found in cat and monkey.

The organization of the vertebrate retina which results in the expression of various cell functions, as outlined above, has not been clearly defined. It is apparent, however, that the concentric center - surround organization is already expressed at the level of the bipolar cell (Naka, 1971; Kaneko, 1973; Werblin, 1974). The antagonistic surround of bipolar cells is large, eg. 1 - 1.5 mm in diameter in goldfish (Kaneko, 1973). This is greater than the dendritic spread of the bipolar cell and, therefore, a lateral

spread of information via horizontal cells is probable. Werblin and Dowling (1969) showed in mud puppy that bipolar cells can be activated by two specific pathways. First, a small spot of light centered on the photoreceptors directly connected with the dendritic field of the bipolar cell will selectively activate the receptor to bipolar pathway. Second, stimulation of an area surrounding the bipolar receptive field will activate receptors that drive horizontal cells in contact with the bipolar. The basic structure of the concentric receptive fields, therefore, is established by three neural elements: the receptors, the horizontal cells and the bipolar cells (Naka, 1976).

The more complex receptive field organization seen at the level of the ganglion cell is less clear. It has been suggested, however, that the classification of ganglion cells into tonic and phasic types may be dependent on interactions occurring in the inner plexiform layer (Werblin and Copenhagen, 1974). The activity of the tonic ganglion cell resembles that of the bipolar, while the activity of the phasic ganglion cell resembles that of the amacrine cell. It is conceivable that the division of ganglion cells into these two types is dependent on whether they receive most of their input from bipolar cells or from amacrine cells. The organization of such properties as directional sensi-

tivity may also occur within the inner plexiform layer and be dependent on amacrine cell activity (Wyatt and Daw, 1976).

The axons of ganglion cells make up the optic nerve and terminate in more central regions of the brain. In the cat, these fibers project to five regions of the brain: 1) the dorsal lateral geniculate nucleus, 2) the ventral lateral geniculate nucleus, 3) the pretectum, 4) the superior colliculus and 5) the accessory optic tract nuclei (Rodieck, 1973). This pattern appears to be common to most species. In mammals, the dorsal lateral geniculate represents the major terminal for retinal fibers and the visual cortex receives its major input from this nucleus. In non-mammalian species, most ganglion cell axons terminate in the optic tectum which is the major visual center for these animals (Duke-Elder, 1958).

Processing of visual information in the retina is dependent on the integrated activity of all the neural cell types described above. One approach to the elucidation of the mechanisms involved, therefore, is the study of the retina functioning as a unit. The following sections describe some of the electrophysiological responses of the whole retina and various aspects of visual function which depend on the interaction of differing neuronal elements.

The electroretinogram

Holmgren (1865) observed that the difference in electrical potential measured across the living eye could be altered by light stimulation. The discovery of the electroretinogram (ERG), which is the term used to identify this gross potential change, is generally attributed to him. Early efforts were aimed at discovering the site of origin of these potentials. The retina was implicated early and recordings obtained from the isolated retina gave conclusive evidence to support this theory (Kuhne and Steiner, 1881). The electroretinograms obtained directly from the frog eye by Einthoven and Jolly (1908) are of classic importance because they were the first to demonstrate the various major components of the electroretinogram recognized today. These authors observed that illumination of the eye produced an initial cornea negative potential, followed by an upward positive deflection which was itself followed by a second positive response. They attributed these waves to the successive reactions of three substances in the retina, which they termed simply "A", "B" and "C". They presumed that the changes that light made in each gave rise to a characteristic electrical response. The terms a-, b- and c-waves of electroretinography found their origin here.

Piper (1911) factored the electroretinogram into three parts. The first two components were labelled I and

II and were described as continuous activities which were present as long as the stimulus stayed on. He attempted to obtain supportive evidence for his analysis from comparative studies. He observed that the relatively simple retina of the cephalopod produced a simple response to stimulation which could be matched with his first component. By subtracting this first component from the electroretinographic waveform found in birds and amphibians, he obtained his second component. These first two components appeared with different latencies and rates of development and their interaction produced the a- and b-waves. Some of his recordings showed a c-wave and his third component could be obtained by taking the difference between records having the c-wave and those which did not. Although the conclusions drawn from these studies are highly speculative, the idea that the electroretinogram is the result of several components which add together to produce the final waveform laid the basis for much subsequent work (Armington, 1974).

The most important analysis of the ERG was done by Granit (1933). His study has served as the basic model for the interpretation of the electroretinogram for over 40 years. Granit realized that it was possible to resolve a complex curve like the ERG in an infinite number of ways, but that no good interpretation could be obtained until this

was done by biological means. His work was based on the premise that the cells of the retina, which had become differentiated into various types, would probably show selective sensitivity or resistance to various agents. In these experiments ether was chosen as the differentiating agent and was administered by inhalation to the decerebrate cat preparation. After the initiation of ether anesthesia, the rising phase of the c-wave was lost first while the faster components appeared to be unaltered. This component, which is easily removed by narcotization, he termed the first process or PI. Further administration of ether led to the disappearance of the remaining positive potential, which he termed PII, and left a purely negative potential termed PIII. The final waveform of the ERG is made up of the algebraic summation of these processes. The rising phase of the c-wave is largely due to the slowly developed positive PI. The b-wave represents the first positive component of PII, while the a-wave reflects the leading edge of PIII. Differences in the final waveform could be seen between retinas which are dominated by cones rather than rods, and this was thought to reflect primary differences between rod vs. cone PIII.

Essentially, this interpretation of the electroretinogram has remained useful with few modifications. Brown

(1968) was able to demonstrate that Granit's PII is actually made up itself of two components, a transient positive b-wave and a positive d.c. component. Knave et al. (1972) described both positive and negative d.c. components, while Rodieck (1972) asserted that changes in the amplitude of some of the slow waves of the ERG could account for findings not readily explained using Granit's analysis. These alterations have not substantially changed Granit's model however.

Along with resolving the ERG into its various components, a central problem has always been the identity of the retinal cell type which generates each of these components. Noel (1959), in studies on the rabbit, pointed to the pigment epithelium as the site of origin of the c-wave or PI process. He observed that administration of sodium iodate which causes retinal degeneration primarily of the pigment epithelium caused a disappearance of the c-wave. Furthermore, enhancement of the c-wave by sodium azide could be blocked by sodium iodate, but not by iodoacetic acid, a drug known to cause photoreceptor damage. Later evidence obtained with microelectrode recordings gave support to this hypothesis (Brown and Wiesel, 1961). It is thought that the hyperpolarization seen in pigment epithelial cells is induced by decreases in extracellular K^+ concentration in the receptor layer (Oakley and Green, 1976).

The cellular origin of PII was thought to be the bipolar cell layer and studies of the intraretinal ERG suggested that the b-wave generator does, in fact, lie in the inner nuclear layer (Brown, 1968). Miller and Dowling (1970) studied the various retinal cell types by intracellular recording and compared the responses with the ERG. They found that the glial cells of Muller most closely matched the b-wave with respect to latency, waveform and dynamic range. It has been suggested that a local depolarizing potential occurs in Muller cells as a result of light-induced activity in distal retinal neurons, possibly resulting from changes in extracellular K^+ concentrations (Miller, 1973; Karwoski and Proenza, 1977). The radial flow of current through the retina associated with this depolarization is recorded as the b-wave of the ERG.

Granit's PIII process, which represents the a-wave in the intact ERG, is felt to be generated by the photoreceptors. Supportive evidence has been obtained by voltage-depth profiles of the a-wave of the local ERG in cat retina. The a-wave amplitude was largest when the microelectrode was located in the photoreceptor layer (Brown and Wiesel, 1961). Studies involving foveal vs. parafoveal recordings and effects of clamping of the retinal circulation in monkeys (Brown and Watanabe, 1962) have given further support to the

idea that the a-wave of the ERG represents photoreceptor activity. In cold-blooded vertebrates the PIII process has been divided into two components, proximal and distal PIII (Murakami and Kaneko, 1966). It was demonstrated that the distal PIII arises from the receptor cell layer and that the proximal PIII arises from structures lying more vitread. Proximal PIII has a longer latency, so that the a-wave of the ERG represents the leading edge of distal PIII.

The electroretinogram represents contributions from both the rod and cone systems, therefore, the final waveform is a result of the photoreceptor composition of the retina under study. Granit (1935) classified retinas under two headings, E retinas and I retinas. The I retinas were characterized by more labile and brisk responses, and, after a species comparison, he suggested that the E type retina was more typical of rod-dominated nocturnal animals while the I type retina was associated with more cone-dominated diurnal species. In mixed retinas, such as in frogs, the final waveform is dependent upon the stimulation parameters used. When the retina is dark-adapted and stimulated with dim test flashes, the electroretinogram has a small a-wave and a smooth b-wave, behaving as an E retina. If the retina is light-adapted, however, and stimulated with bright test flashes, the a-wave deepens and the b-wave is small and

pointed, resembling an I retina.

Another characteristic difference between responses from rod- and cone-dominated retinas occurs at the termination of the test flash. In rod-dominated retinas there is a small negative change termed the off-response, while in cone-dominated retinas there is a large, transient positive potential termed the d-wave. These variations are thought to be due to differences in decay rate of the receptor potentials. In the rod-dominated retina, the end of stimulation is followed by a fast decline of the positive d.c. component of PII and a slower decay of the negative late receptor potential. The result is a slight negative shift in the ERG. In the cone-dominated retina, the negative late receptor potential decays much faster at the end of stimulation than the positive d.c. component, which leads to a large positive deflection of the ERG.

In addition to the major components described above, a number of other components contribute to the detailed form of the ERG. At high stimulus intensities, for example, rapid oscillations may be seen superimposed on the b-wave in some species (Brown, 1968). The early receptor potential (ERP) is a fast, biphasic response which precedes the ERG and which has been shown to be generated primarily by cones (Goldstein, 1967). The relationship between these and other field po-

tentials generated by light stimulation and the processing of visual information in the retina is not fully understood.

Delayed off-responses

Another component of the ERG which has received some attention is the e-wave. Sickel and Crescitelli (1967) observed long-delayed responses to light stimulation in the isolated frog retina. Slow potential changes were elicited; the latency of which lengthened with increasing stimulus intensity, differing from that of other ERG components. Associated with these slow potential changes was a burst of spike discharges which centered around the maximum change of the slow wave. These slow positive potentials are off-effects since they have been shown to be linked to the cessation of light stimulation (Crescitelli and Sickel, 1968). The amplitude and latency of this response are associated with the history of light treatment of the retina. The delayed off-responses are also seen in unbleached retinas, but in such preparations their appearance is more variable and their amplitude smaller (Crescitelli and Sickel, 1968). However, bleaching of the retina for periods up to 30 minutes (at intensities which reduce rhodopsin concentration by as much as 95 - 97%) allows for the development of large delayed off-responses during subsequent dark-adaptation (Crescitelli and Sickel, 1968). The action spectrum for

this response corresponds to the rhodopsin absorbance curve suggesting that this delayed off-response is mediated via a rhodopsin rod mechanism (Crescitelli and Sickel, 1968). It was suggested that this delayed off-response, or e-wave, is the result of a release from long-acting photopic inhibition, demonstrating an interaction between cone and rod activity (Crescitelli and Sickel, 1968). A subsequent observation in the eye of a tadpole (at a stage when all responses to light are negative) that a cornea negative response exists with a time-course and latency similar to that of the e-wave in adult frogs gives support for the idea that the delayed off-response is associated with receptor activity (Crescitelli, 1970).

A similar long-delayed response to light stimulation has been observed in frog retinal ganglion cells (Pickering and Varju, 1967). It was demonstrated that a burst of ganglion cell activity occurred after light stimulation with a latency which was related to stimulus intensity. Increasing stimulus intensity produced a longer latency of the delayed response (Pickering and Varju, 1967; Pickering, 1968; Varju and Pickering, 1969). Furthermore, the latency of this response was influenced by two other parameters: 1) background illumination and 2) the degree of dark-adaptation. Latency increased as steady background illumination de-

creased (Pickering and Varju, 1969) and as dark-adaptation (measured by total time in darkness) increased (Pickering, 1968). It was suggested that the latency of the delayed response is an expression of the interaction between excitatory and inhibitory processes initiated in the retina by light stimulation (Varju and Pickering, 1969). After studying the response characteristics of on-off ganglion cells, it was postulated that the excitatory process seems to be primarily related to background illumination and level of adaptation, while the inhibitory process seems to be more a function of the intensity of the on-stimulus (Pickering and Varju, 1971). The final expression of this interaction is the latency of the delayed-response and the duration of the train and total number of ganglion cell discharges recorded. The similarity between this delayed response and the e-wave described by Sickel and Crescitelli suggests that these two responses are manifestations of the same phenomenon (Pickering and Varju, 1971; Crescitelli and Sickel, 1968).

Light and Dark Adaptation

Adaptation is that property of the visual system which allows the eye to function in widely changing levels of illumination. This range of ambient light levels may be as great as 7 log units. The adaptation mechanism has

been thought to involve visual pigment metabolism and/or changes in the neural apparatus of the retina. Historically, investigators have studied only one aspect of adaptation, namely the process of dark-adaptation. In these studies the sensitivity of the retina to dim test flashes was examined after exposure to bright light. A decrease in threshold occurred over a long time-course, with approximately 45 minutes required to reach maximum sensitivity in man (Piper, 1903). It was found that the curve relating the logarithm of retinal sensitivity to time was divisible into two sections, corresponding to changes in cone vs. rod sensitivity (Kohlrausch, 1922). Hecht (1920, 1937) postulated that photochemical changes alone were involved in visual adaptation. He suggested that levels of a photosensitive substance and its two products of decomposition were entirely responsible for conditioning the visual system to operate at various levels of illumination. The time-course of dark-adaptation depends, therefore, on the course of a "dark" reaction, during which two products of decomposition reunite to synthesize the original photosensitive substance. Some studies have offered support for this hypothesis by demonstrating a very close correlation between the amount of visual pigment present and the degree of sensitivity of the retina (Dowling, 1960; Rushton, 1961). However, the

relationship between photopigment concentration and sensitivity is not a simple one. Serious discrepancies arise when one attempts to relate the decrease in threshold during dark-adaptation to an increasing concentration of visual pigment. The changes in visual pigment concentration are not nearly enough to account for the observed alterations in sensitivity (Lythgoe, 1940; Campbell and Rushton, 1955; Dowling, 1963). Furthermore, if retinal sensitivity depended entirely on photopigment concentration, then the recovery curves following light-adaptation should be able to be superimposed if they start from the same concentration of photopigment. This was observed not to be so and, in fact, these recovery curves have been shown to depend on such adapting light parameters as size and duration of exposure (Lythgoe, 1940; Wright, 1946). A mechanism whereby some of these problems could be answered was postulated by Wald (1954). He suggested that dark-adaptation could still be related to receptor desensitization if the threshold of a dark-adapted rod depended upon its entire content of rhodopsin. The rod is a compartmented structure and could undergo, compartment by compartment, a stepwise response. In each compartment any molecule of rhodopsin which absorbs a quantum of light would discharge the compartment. Therefore, a large rise in threshold is achieved with very

little bleaching of rhodopsin. A discharged compartment could not contribute further to a response until all of its rhodopsin is restored. The remaining rhodopsin of a discharged compartment absorbs light just as before, but this absorption has no effect other than to delay the eventual recovery of the compartment. By modification of the number and size of compartments postulated, this theory could be made to relate threshold to visual pigment concentration fairly well (Brindley, 1970).

Another approach to dark-adaptation was explored by Barlow (1964). He postulated that bleaching light raises the background of spontaneous activity so that previously detectable signals become lost in noise. It is the slow decline of this noise following light adaptation that allows for the decrease in threshold observed during dark-adaptation. The level of background noise is dependent on the amount of photopigment bleached and would be indistinguishable from that produced by light. Objections to this hypothesis have been raised, however, by studies which demonstrate that light-adaptation and background light effect visual processing differently and are, therefore, not indistinguishable (Rushton, 1965; Ernst, 1968; Naka and Rushton, 1968).

Although it appears that after bleaching of visual

pigments the recovery of sensitivity depends on pigment regeneration, the relationship between very weak background light and retinal sensitivity is not clearly understood. Under conditions where no significant fraction of visual pigment is bleached there is still observed a large increase in visual threshold. Dowling (1963) observed in rats that decrease in visual threshold was very rapid following light-adaptation which bleached little rhodopsin. At higher background intensities, where rhodopsin concentrations were significantly reduced, the recovery curves were divided into an initial rapid phase followed by a slower phase leading to recovery of dark-adapted threshold. The slow recovery phase occurs in parallel with the regeneration of rhodopsin. The initial fast component is not related to visual pigment levels and is referred to as "neural" adaptation. This rapid or "neural" adaptation mechanism was also demonstrated in frog (Frank, 1971; Sillman et al., 1973), skate (Dowling and Ripps, 1972) and carp (Witkovsky et al., 1973).

Furthermore, except for Barlow's hypothesis, theories relating visual threshold entirely to receptor desensitization are unable to account for one very important observation, and that is that adapting light falling on the retina peripheral to the test stimulus is still able to alter its threshold (Schouten and Ornstein, 1939; Wright,

1946; Kaplan and Ripps, 1960; Lipzet, 1961; Easter, 1968). Rushton and Westheimer (1962) demonstrated that bleaching of some rods could increase the threshold for test stimuli to neighboring unbleached rods. These observations are difficult to reconcile with a purely photochemical theory of light- and dark-adaptation. Furthermore, it has been observed that light- and dark-adaptation not only involves changes in threshold but also changes in the organization of neural elements in the retina (Arden and Weale, 1954; Barlow et al., 1957). These studies have led to the belief that alterations in retinal sensitivity involve two mechanisms, one dependent on photopigment levels and the second involved with neuronal organization (Thomson, 1950; Arden and Weale, 1954; Dowling, 1963; Rushton, 1965b).

It is the localization of the site of neural adaptation which has involved numerous investigators in recent years. John Dowling (1967), studying visual adaptation in the rat, postulated that neural mechanisms operating in the bipolar cell layer were responsible for controlling retinal sensitivity by some form of negative feedback. This idea was supported by morphological studies which showed the existence of reciprocal synapses in this neuronal layer (Dowling and Boycott, 1965; Dowling and Boycott, 1966).

Later studies revealed, however, that so-called "neural" adaptation may be related to mechanisms other than bleaching which are operating in the photoreceptors themselves. Boynton and Whitten (1970) observed in monkeys that background light produces a steady receptor response to which a test flash must add. They proposed that this steady voltage accounts for the loss in gain that accompanies exposure to background light. That is, background light pushes the receptor towards its maximal response, thereby compressing the range over which responses can be generated. At high intensities of background light, bleaching of visual pigment reduces the rate of quantal absorption thereby lowering the amplitude of the potential generated by background light to below saturation levels. This would effectively extend the range of the receptor response. In a series of studies on adaptation in the skate, Dowling and Ripps (1970; 1971; 1972) observed that photoreceptors exhibited the full range of adaptational changes observed in more proximal cells leading them to postulate that visual adaptation in the skate is governed primarily by the photoreceptors themselves. Grabowski et al. (1972), recording intracellularly from rods in Axolotl, showed an increased sensitivity of rods following bleach which was not related to photopigment regeneration. Frank (1971) demonstrated "neural" adaptation

in the PIII component of the frog ERG, indicating that this fast component of adaptation is not restricted to cells proximal to the receptors. These results were confirmed by Hood *et al.* (1973).

The studies above have all examined the effects of average background illumination on the response characteristics of various retinal cells measured intracellularly, or extracellularly as unit responses or as mass potential changes. In a series of papers, Werblin and co-workers demonstrated that sensitivity control may be more complex than the aforementioned studies would indicate. In the mixed rod-cone retina of *Necturus* it was shown that alterations in threshold produced by average background illumination are derived primarily from mechanisms operating in the photoreceptors themselves (Normann and Werblin, 1974). The photoreceptor response range can be shifted as much as 7 log units by background light. At the level of the second order neuron, or bipolar cell, responses to center test flashes can be further altered by presentation of surround background illumination (Werblin, 1974). At the ganglion cell level, further modifications of responses are dependent on moving background light (Werblin and Copenhagen, 1974). All three mechanisms combine to adjust retinal sensitivity to ambient light conditions and to control retinal output. The last

two mechanisms are dependent on synaptic activity occurring at the outer and inner plexiform layers, respectively.

In a recent study by Green et al. (1975) in the skate, it was demonstrated that even full field background illumination could involve mechanisms operating proximal to receptors if the background light was very dim. Under these conditions thresholds of both the b-wave and the ganglion cell were raised significantly even though there was no measurable effect on the sensitivities of either the isolated receptor potential or the S-potential. Their results suggested the presence of a "network" mechanism of adaptation in the retina which can significantly effect b-wave and ganglion cell activity but which behaves independently of the receptors and horizontal cells. Brighter backgrounds, on the other hand, exert their influence on retinal sensitivity by receptoral mechanisms.

If photoreceptors themselves are involved in the "neural" phase of adaptation, then some mechanism other than visual pigment levels must account for this control of retinal sensitivity. Donner and Reuter (1967), studying dark-adaptation in excised frog eyes, demonstrated that ganglion cell threshold following bleach decreased along a curve having a fast and slow component. The fast or "neural" component had the same time-course as the decay of the rhodopsin

photoproduct, metarhodopsin II. They postulated that metarhodopsin II desensitizes the photoreceptor and that the rapid increase in sensitivity seen during the early phase of dark-adaptation is due to the decay of this photoproduct. This idea was supported by Baumann and Scheibner (1968), whose study on ganglion cell threshold in isolated frog retina also showed a correlation between recovery curves during dark-adaptation and the decay of metarhodopsin. Conflicting results were obtained in the isolated rat retina by Frank and Dowling (1968). They observed alterations in b-wave threshold after flash bleach which were not related to the decay of metarhodopsin or a 467 nm intermediate photoproduct. Similarly, Frank (1971) observed in isolated frog retinas that the mass receptor potential demonstrated a neural adaptation component of its dark-adaptation curve which was not influenced by changes in temperature, an observation incompatible with the theory that neural adaptation is dependent on the decay of rhodopsin photoproducts. Recently, however, there has been demonstrated in rods (Sillman et al., 1973) and cones (Sillman, 1974) a rapid dark-adaptation which is postulated to be related to the decay of a rhodopsin photoproduct, such as metarhodopsin II, so that more work is needed to clarify this problem.

Another interesting possibility for a "neural" adap-

tation mechanism in the photoreceptors may involve cyclic nucleotides. It has been observed that retinal guanosine 3',5' - monophosphate (cyclic GMP) concentrations decrease after exposure to light (Bitensky et al., 1971; Goridis et al., 1974). A high cyclic GMP phosphodiesterase activity has been found in purified outer segments (Chader et al., 1974) and the decrease in cyclic GMP levels has been accounted for by the light activation of this phosphodiesterase (Miki et al., 1973; Goridis and Virmaux, 1974). Because of the involvement of cyclic nucleotides in regulation of sodium permeability (DeLorenzo et al., 1973) and neuronal excitability (Hoffer et al., 1972; Stone et al., 1975), it has been postulated that cyclic nucleotides, in particular cyclic GMP, may function in the regulation of photoreceptor sensitivity during light- and dark-adaptation (Bitensky et al., 1973; Goridis et al., 1976). However, there has been no physiological evidence for cyclic nucleotide involvement in receptor recovery following adapting light (Hood and Ebrey, 1974) so that no conclusions can be realistically drawn about the role of cyclic nucleotides in "neural" adaptation.

Mechanisms for "neural" adaptation at sites other than the photoreceptors are even less well established. In studies on the rat, Graham and Pong (1972) have demonstrated

alterations in b-wave threshold by GABA and GABA antagonists bicuculline and picrotoxin under light adaptation conditions which had no effect on photoreceptor responses. It was postulated that GABA may be involved in an automatic gain control mechanism activated by background illumination (Graham and Pong, 1972; Graham, 1974). Under similar conditions of light adaptation, Dowling and Ripps (1976) demonstrated a relationship between extracellular potassium concentration and alterations in sensitivity produced by background light that led them to suggest the possibility that alterations in $[K^+]_o$ may form the basis of network adaptation.

Retinal neurotransmitters

The morphology of retinal synapses, even the specialized ribbon synapses associated with photoreceptor and bipolar cells, suggest the presence of chemical transmission in the retina. The following paragraphs outline evidence supporting various agents as possible retinal neurotransmitters.

1) Acetylcholine

The acetylcholine (ACh) content of vertebrate retinas is approximately 30 nmoles/g wet weight (Graham, 1974). There have been no studies, however, on the content of ACh in specific retinal layers. The retina is one of the richest sources of the enzyme choline acetyl transferase (Hebb, 1955) and in studies on pigeon, rat, frog and mudpuppy it

was shown that the highest enzyme activity was concentrated in the inner plexiform layer with little activity associated with receptor or horizontal cell fractions (Graham, 1974). Different results were obtained in turtle where it was shown that photoreceptors were able to synthesize acetylcholine but not GABA or dopamine (Lam, 1972a). More recent findings, however, that the specific activity of choline acetyl transferase in turtle photoreceptors is only about 10 - 30% of that found in whole retina weaken the hypothesis that the receptors are cholinergic (Lam, 1975).

The activity of acetylcholinesterase (AChE) in the retina characteristically is concentrated in the inner plexiform layer. Koelle et al. (1952) showed that specific AChE activity was localized in the cell bodies and processes of amacrine cells in the cat, and this was later extended to include pigeon, squirrel, rabbit and rat (Nicholls and Koelle, 1968). However, Reale et al. (1971) were able to demonstrate some AChE activity associated with the outer plexiform layer in rabbit. It has recently been observed that the retina shows specific binding for α -bungarotoxin, an indication of the presence of nicotinic receptors (Vogel and Nirenberg, 1976; Wang and Schmidt, 1976). With radioautography utilizing labeled α -bungarotoxin, nicotinic receptors have been demonstrated in both the outer and inner

plexiform layers of goldfish and turtle (Yazulla and Schmidt, 1976). This would offer evidence in favor of the presence of cholinoceptive neurons in both synaptic layers.

In an attempt to relate ACh to a physiological function in the retina, ACh content was measured after light- or dark-adaptation. However, no significant differences in ACh content were demonstrable between these two conditions (Easton, 1945; Graham, 1974). Similarly, very little difference in AChE activity was seen between light- and dark-adapted retinas (Graham, 1974).

The influence of cholinergic agents on retinal function has been studied. Noell and Lasansky (1959) demonstrated retinal ganglion cell excitation in cat and rabbit by electrophoretically applied ACh. Straschill (1968) observed an increase in cat retinal ganglion cell activity after intra-arterial injection of ACh, however, neither atropine nor dihydro-β-erythroidin interfered with spontaneous or light-driven activity. With inotophoretically applied drug, it was later observed that ACh had an excitatory effect on off-center ganglion cells and an inhibitory effect on on-center neurons (Straschill and Perwein, 1973). In the isolated retina of the rabbit, it was shown that physostigmine increased both spontaneous and evoked activity of on- and on-off ganglion cells (Ames and Pollen, 1969).

In the same study d-tubocurarine was shown to depress both spontaneous and light-evoked activity, while atropine, generally, was without effect. ACh has no effect on intracellularly recorded potentials of photoreceptors or horizontal cells (Murakami et al., 1972). The influence of cholinergic agents on neurons of the inner nuclear layer can be studied by an examination of the mass potential changes of the ERG. Von Bredow et al. (1971) observed in the cat a large increase in b-wave amplitude after the intra-arterial administration of Sarin (isopropyl methylphosphonoflouridate), which could be reversed by the injection of atropine. ACh produces a rapid, transient increase in b-wave amplitude, while that after carbachol was longer in duration. Although the evidence cited above argues for the presence of cholinergic synapses in the vertebrate retina, it is not now possible to specify the sites of cholinergic neurons nor to indicate their possible role in retinal function.

2) Catecholamines

The development of the Falk - Hillarp histochemical technique for the localization of catecholamine containing neurons provided major impetus for the study of these agents as possible retinal neurotransmitters. Using this technique, Malmfors (1963) demonstrated the presence of fluorescent cell bodies in the inner nuclear layer and fibers in the

inner plexiform layer of the rat, which he believed represented catecholamine containing neurons. Haggendal and Malmfors (1963), using a biochemical approach, showed that dopamine was the dominant catecholamine in the retina of rabbits. They believed that retinal dopamine was probably localized in the catecholaminergic neurons demonstrated by the fluorescent histochemical procedure. Dopamine containing neurons with cell bodies in the inner nuclear layer and processes in the inner plexiform layer were later identified in the rabbit retina (Haggendal and Malmfors, 1965). Reserpine caused a disappearance of fluorescence in these preparations while sympathetic denervation had no effect. The presence of catecholamine containing neurons has since been demonstrated in the retinas of numerous species: fish (Ehinger et al., 1969), frogs (Ehinger, 1976), birds (Ehinger, 1967) and monkeys (Laties and Jacobowitz, 1966; Ehinger and Falk, 1969).

Cells with their perikaryia in the amacrine cell layer and processes extending into both the inner and outer plexiform layers have been described using the Golgi method (Gallego, 1971; Dawson and Perez, 1973; Boycott et al., 1975). These cells have been termed "interplexiform" cells and it has been postulated that they may serve in some feedback mechanism operating within the retina (Gallego, 1971).

Recently, these interplexiform cells have been shown to be dopaminergic neurons in the goldfish and monkey (Dowling and Ehinger, 1975; Dowling et al., 1976). Since the action of dopamine is generally inhibitory in the vertebrate retina, it is felt that these cells may form centrifugal pathways within the retina and regulate center - surround antagonism at the outer plexiform layer (Dowling et al., 1976). The failure to demonstrate catecholamines in cat interplexiform cells suggest that this cell type may be a general feature of vertebrate retinas but that different species may use different neurotransmitters (Dowling et al., 1976).

In an attempt to relate catecholamines to visual function some studies have examined the relationship between retinal catecholamine concentrations and conditions of light- and dark-adaptation. Drujan et al. (1965) observed a decrease in dopamine content in retinas of frog, toad and rabbit after light-adaptation. Similarly, light has been shown to cause a depletion of epinephrine in toad retina (Drujan and Diaz Borges, 1968). In contrast, Nichols et al. (1967) reported an increase in retinal dopamine after exposure to light in rabbit, rat and guinea pig. This discrepancy is difficult to understand, but it may be related to differences in methods of light-adaptation and/or in procedures of retinal tissue isolation. Drujan and co-workers

apparently used a lower intensity when adapting their animals and required a longer time in isolating the tissue after death of the animal. How these differences would effect catecholamine content of the retina is not clear, however. Both groups of workers concluded that their data pointed to an increased activity of catecholaminergic neurons.

The application of exogenous dopamine has been shown to depress spontaneous activity in retinal ganglion cells of rabbit (Ames and Pollen, 1969) and cat (Straschill and Perwein, 1969; Straschill and Perwein, 1975). In light-evoked responses excitatory reactions were depressed and inhibitory events were augmented (Ames and Pollen, 1969; Straschill and Perwein, 1969). Dopamine has also been observed to depress light-evoked responses of goldfish horizontal and bipolar cells, while having no significant effect on photoreceptor activity (Dowling et al., 1976). The intravenous administration of L-DOPA has been shown to cause a dose dependent decrease in the light-evoked optic tract potential in cat, while the b-wave of the ERG remained unaffected (Straschill and Perwein, 1969; Straschill and Perwein, 1975). In contrast, Starr (1975b) reports reduced amplitudes of both a- and b- waves of the rabbit ERG after administration of dopamine to an eye-cup preparation.

Kramer and co-workers reported a series of experi-

ments designed to offer evidence concerning dopamine's role as a possible neurotransmitter. They demonstrated the release of dopamine in the cat following light stimulation and that this rate was dependent on the rate of stimulation (Kramer, 1971). Furthermore, a greater release of dopamine was observed in light-adapted preparations than in dark-adapted animals. Utilizing radioautography, it was shown that the administration of ^{3}H - dopamine by the pre-retinal perfusion technique used in this study led to the accumulation of dopamine in neurons corresponding exactly to the catecholamine-containing neurons seen with the Falk - Hillarp method (Kramer et al., 1971). Following carotid infusion, little dopamine was localized in the retina, suggesting a significant blood-retinal barrier to dopamine (Kramer et al., 1971).

3) GABA

γ - aminobutyric acid (GABA) is present in high concentrations (1.5 - 3mM) within the vertebrate retina (Van Harrevald, 1976). This amino acid is not evenly distributed throughout the retina but appears to be concentrated in proximal regions (Cohen et al., 1973; Kennedy and Voaden, 1974). In particular, the highest concentrations in rabbit retina have been reported in the ganglion cell layer (Kuriyama et al., 1968) and in frog in the inner synaptic layer

(Graham, 1972).

An uptake mechanism for GABA has been demonstrated in the retina (Goodchild and Neal, 1970) and many studies have shown an accumulation of exogenous GABA by retinal cells. The uptake of tritiated GABA was shown to be localized mainly in the inner plexiform layer of rabbit (Ehinger, 1970), while in goldfish it is primarily the horizontal cells which show this accumulation (Lam and Steinman, 1971). Further studies have indicated that the uptake of GABA into retinal tissues shows marked species differences. In mammalian species, such as rat, guinea-pig, goat and cat, GABA accumulation is restricted primarily to Muller cells (Marshall and Voaden, 1975). In contrast, species such as frog (Voaden et al., 1974), goldfish (Lam and Steinman, 1971), pigeon and chicken (Marshall and Voaden, 1974) show uptake solely in retinal neurons. These two patterns of uptake reflect the general type of retinal organization found in each group of animals, namely simple and complex retinas, respectively. This may indicate a fundamental difference in the way in which these two groups handle the amino acid.

Enzymes for the synthesis and degradation of GABA exist within the retina (Kuriyama et al., 1968; Graham, 1972), and the rate of synthesis of this amino acid has been shown to be influenced by light stimulation (Lam, 1972b;

Starr, 1975a). Furthermore, release of GABA from retinas preloaded with ^{3}H-GABA has been reported. This efflux of GABA has been demonstrated in response to electrical stimulation and K^{+} depolarization in rat (Voaden and Starr, 1972) and in response to light stimulation in rabbit (Bauer and Ehinger, 1977a).

Application of exogenous GABA has no appreciable effect on photoreceptor activity (Murakami et al., 1972). Horizontal cells, however, are hyperpolarized by GABA and their response to light suppressed (Murakami et al., 1972; Sugawara and Negishi, 1973a). Both spontaneous and evoked activity of ganglion cells are depressed by GABA (0.1mM) in isolated rabbit retina (Ames and Pollen, 1969). Similarly, light induced ganglion cell discharges in frog retinas are also reduced by 0.1 to 10mM GABA (Kishida and Naka, 1968). The iontopheretic application of GABA to retinal ganglion cells inhibited spontaneous and light-evoked activity in rabbit (Noell, 1959) and cat (Straschill and Perwein, 1969).

Exogenous GABA has also been shown to influence retinal field potentials markedly. Scholes and Roberts (1964) observed that intravenously administered GABA caused a depression of the ERG in chickens at low stimulus intensities and an enhancement of both a- and b-waves at high stimulus intensities. Pasantes-Morales et al. (1973) observed a

depression of b-wave amplitude at all stimulus intensities studied in chicken after intravitreal injection of GABA. Picrotoxin produced a transient reversal of this depression (Bonaventure et al., 1974). Opposite findings have been reported in rabbit where 1mM GABA applied to the *in situ* eye-cup preparation led to an increase in b-wave amplitude but no change in the a-wave of the ERG (Starr, 1975b). In the isolated, perfused frog retina 0.2 mM GABA depresses b-wave amplitude and this depression can be reversed by picrotoxin (Urban et al., 1976).

All of these findings point to the possible role of GABA as a neurotransmitter in the retina. The role of GABA and GABA-containing neurons in visual processes, however, is yet to be clearly defined. Graham *et al.* (1968) observed that GABA levels were higher in light-adapted retinas than in dark-adapted retinas, and that the decrease in GABA concentration during dark-adaptation was very slow, requiring about 2 hours. Very similar results were obtained in goldfish where Lam (1972) showed that retinal GABA levels increase slowly in light and decrease slowly in dark. Furthermore, he showed that the light induced increase in GABA was proportional to the intensity of illumination used. These studies point to the possible role of GABA in light- and dark-adaptation. Studies of increment threshold curves

in rat have shown that GABA decreases and picrotoxin increases retinal sensitivity at low levels of background illumination (Graham, 1974). It was suggested that GABA is modifying retinal sensitivity by altering a negative feedback mechanism involved in an "automatic gain control" system. It has been suggested that this type of process is involved in adjusting the retina to varying levels of background light (Rushton, 1965b).

A local, light-evoked extracellular potential, the proximal negative response (PNR), has been described in frog retina (Burkhardt,1972). This PNR has been associated with amacrine cell activity and it was observed that picrotoxin increased the amplitude but not the latency of this response. Since the morphology of the bipolar-amacrine synapse suggests the possibility of negative feedback, Burkhardt argued that the increase in amplitude of the PNR, without a change in latency, was due to an attenuation of recurrent inhibition by picrotoxin.

The GABA system may also be involved in the receptive field organization of retinal ganglion cells. Wyatt and Daw (1976) demonstrated that picrotoxin administration abolished directional sensitivity in rabbit ganglion cells, while strychnine, an antagonist of glycine, had no such effect. Kirby and Enroth-Cugell (1976) observed in cat that

intravenous administration of picrotoxin or bicuculline reduced the surround component of the center-surround organization of y-cells. Responses of x-cells remained virtually unaffected by either drug.

The observations above strongly suggest that GABA has a neurotransmitter role in the vertebrate retina. Its involvement in light- and dark-adaptation and/or receptive field organization (processes probably mediated by the lateral spread of visual information) would be in keeping with the localization of this amino acid in the inner and outer plexiform layers.

4) Glycine

Glycine has also been shown to exist in high concentrations in the vertebrate retina (Pasantes-Morales et al., 1972; Cohen et al., 1973). Furthermore, this amino acid does not appear to be evenly distributed but to be concentrated in more proximal retinal layers (Cohen et al., 1973; Yates and Keen, 1976). Glycine is actively taken up by the retina and autoradiographic studies have demonstrated that labelled glycine is concentrated in certain cells, mainly in the position of amacrines (Bruun and Ehinger, 1972; Voaden et al., 1974). Recent observations in the isolated frog retina, however, indicate that ^{3}H - glycine may also be taken up by synaptic terminals of the photoreceptor cells

(Marshall and Voaden, 1976). The functional significance of this is not clear since this localization has not been reported in any other species. Light flashes increase the release of labelled glycine from pre-loaded rabbit retinas, while continuous light has no effect (Bauer and Ehinger, 1977b). Since amacrine cells respond preferentially to changes in illumination, these results are in keeping with the autoradiographic findings.

The administration of exogenous glycine hyperpolarizes horizontal cells and reduces S-potentials, but has no appreciable effect on photoreceptor activity (Murakami et al., 1972). Ames and Pollen (1969) demonstrated that glycine depresses both spontaneous and light-evoked ganglion cell activity, while strychnine, a glycine antagonist, produced an opposite effect. Chu (1968) also observed that strychnine enhanced spontaneous ganglion cell activity in the dark. Furthermore, the "on" inhibition of an off-unit was greatly suppressed by strychnine while the "off" inhibition of an on-unit was not effected. This may indicate a specific role for glycine in the receptive field organization of the ganglion cell.

Glycine also has an inhibitory effect on retinal field potentials. The ERG of the rabbit shows a loss of oscillatory potentials and a reduction of b-wave amplitude

after glycine administration (Korol, 1973; Korol et al., 1975). In the frog there is also a reduction in b-wave with no change observed in a-wave amplitude (Urban et al., 1976). Glycine has the same general effect on retinal responses as GABA but is less potent.

Strychnine effects on the ERG have not lent themselves to an easy interpretation. In the isolated rabbit retina, strychnine at 3.5×10^{-5}M led to an enhanced b-wave, while at 7×10^{-4}M the b-wave was abolished (Vorkel and Hanitzsch, 1971). In the rabbit eye-cup preparation, strychnine at 1mM had no effect on the b-wave, while there was a slight potentiation of the a-wave (Starr, 1975b). In the isolated frog retina, 10^{-5}M strychnine had no effect on the ERG (Urban et al., 1976). It is clear that the relationship between strychnine and retinal function needs further investigation.

The presence of glycine in high concentrations in the retina, its localization to specific cell types, and its release by light stimulation and effects on retinal neurophysiology point to glycine as a strong candidate for a neurotransmitter function in the vertebrate retina.

5) Glutamate and Aspartate

Aspartic and glutamic acids have been found in fairly high concentrations within the retina, with glutamate be-

ing the higher of the two (Pasantes-Morales et al., 1972; Yates and Keen, 1976). Unlike GABA and glycine, the acidic amino acids appear to be evenly distributed throughout the retina (Cohen et al., 1973; Yates and Keen,1976). In keeping with this, autoradiographic studies have shown a more diffuse uptake of glutamic and aspartic acids with radioactivity being limited primarily to glial Muller cells (Ehinger, 1972).

Support for the idea that glutamate and/or aspartate may function as neurotransmitters in the retina has come mainly from the effect these amino acids have on electrophysiological responses. Kishida and Naka (1968) demonstrated that administration of aspartate or glutamate gave rise to an increase in the spike discharge of frog retinal ganglion cells. High concentrations (1mM) led to spontaneous firing followed by irreversible termination of spike discharge. These authors also observed that the combination of excitant and depressant amino acids (such as glycine or alanine) resulted in no alteration in the normal discharge pattern of these cells. They suggested that a proper balance of excitatory and depressant amino acids may be important in regulating ganglion cell activity. Ames and Pollen (1969) also observed that glutamate increased spontaneous activity of ganglion cells in the isolated rabbit retina.

Horizontal cells in the carp retina are depolarized by both aspartate and glutamate, resulting in abolition of the S-potentials (Murakami et al., 1972). Photoreceptor activity in these preparations was not altered, however. Similarly, glutamate and aspartate depolarize turtle horizontal cells and reduce their response to light (Cervetto and MacNichol, 1972). Sugawara and Negishi (1973b) observed that these amino acids had little effect on the resting potentials of bipolar or amacrine cells but that the light-induced responses were abolished immediately after application. Murakami et al. (1975) reported, however, that aspartate and glutamate produced a rapid depolarization of off-center bipolar cells and a hyperpolarization of on-center bipolar cells. In both instances responses to light stimulation were abolished.

Furukawa and Hanawa (1955) first demonstrated that aspartate suppressed the b-wave of the ERG and uncovered a large a-wave. Sillman et al. (1969) extended these observations in the frog retina and showed that not only is the b-wave suppressed but also the proximal PIII component of the late receptor potential. The resulting aspartate-isolated mass receptor response consists only of distal PIII and is a good measure of photoreceptor activity. These results are in good agreement with the effects of glutamate

and aspartate on individual retinal neurons described above. The excitant amino acids do not appear to be as potent as the depressant amino acids. Urban et al. (1976) found a depression in b-wave amplitude in frog retina at concentrations of glutamate > 1mM and aspartate > 5mM. Similarly, Starr (1975b) reported no effect of 1mM glutamate on the ERG of rabbit eyecup.

Several other substances have been postulated to be retinal neurotransmitters, but their functional role is even less clearly defined than those agents just described. Taurine has received, perhaps, more attention than others. It is present in very high concentrations in the retina, with most of it found in the photoreceptor layer (Orr et al., 1976; Yates and Keen, 1976). A light-stimulated release of taurine has been demonstrated in frog rod outer segments (Salceda et al., 1977). The functional significance of this is not clear since taurine has been shown to be inhibitory in the retina (Pasantes-Morales et al., 1973; Bonaventure et al., 1974), while it is generally believed that the photoreceptor neurotransmitter is an excitatory substance. The observation that cat photoreceptors degenerate as a consequence of decreased taurine intake (Hayes et al., 1975) may point to a more general metabolic role for this amino acid in these cells. Taurine has been shown to depress b-wave

amplitude in both rabbit (Starr, 1975b) and frog (Urban et al., 1976), however, and to be more potent than either GABA or glycine.

PURPOSE AND OBJECTIVES

Visual adaptation, i.e. the change in retinal sensitivity with alterations in background illumination, has been shown to be related both to the metabolism of photopigment and to changes in neuronal activity within the retina. The neuronal mechanisms might operate at the level of the photoreceptors or more proximally at the outer and/or inner plexiform layers. It will be the purpose of this study to distinguish between receptor and non-receptor components of adaptation and to assess the role of various putative neurotransmitters in controlling retinal sensitivity.

Visual adaptation mechanisms will be evaluated by observing alterations produced by background light in waveform and stimulus - response curves of various components of the frog electroretinogram. The influence of several putative neurotransmitters, and their antagonists, on these parameters will also be examined and the possible functional role of these agents in the vertebrate retina will be discussed.

METHODS

General

All experiments were performed on isolated retina preparations from frogs (Rana pipiens, southern). These animals had an average body length of 3.5 inches. They were maintained in a tank which had water and dry areas, in a room with a controlled lighting schedule (8 hours light - 16 hours dark). Illumination was provided by full-spectrum fluorescent bulbs (Vitalite) which had a measured intensity of 35 fc at the position of the holding tank. The frogs were adapted to these conditions for at least one week prior to the experiments.

Isolated retina preparation

The isolated retina was chosen for this study because rhodopsin will not regenerate in significant amounts in the absence of pigment epithelium (Rodieck, 1973). This eliminates changes in retinal sensitivity due to photopigment metabolism and makes the analysis of alterations in retinal sensitivity less complicated. Furthermore, the isolated retina preparation is free of influence coming from more central regions of the brain.

Retinal isolation was performed between 1100 and 1300 hours in order to minimize variations due to circadian

rhythms. Animals were dark-adapted overnight and double-pithed under dim background light. The overlying skin and nictitating membrane were removed from around the globe. The extraocular muscles and optic nerve were then cut with a scissors, freeing the eye. After enucleation, the retina isolation procedure was carried out using a dissecting microscope and dim red light (1.0 fc) illumination. The eye was hemisected along the limbus with small scissors. The anterior portion of the eye was removed, together with the lens and adhering vitreous. The retina - choroid complex was gently teased from the sclera by means of blunt forceps. The choroid and retina were then separated, thus isolating the retina. In the dark-adapted state frog photoreceptors have contracted from the pigment epithelium permitting a relatively easy separation. However, in light-adapted animals, the photoreceptors interdigitate with the pigment epithelium cells, making separation of the two layers more difficult. Retinal tears or loss of deep salmon color were reasons for rejection of the tissue.

The retina was placed on an insulated wire mesh receptor side up, and these were then placed in a perfusion chamber. The chamber, made of black plexiglas, had a tissue compartment with the following dimensions: 6.0 cm x 1.5 cm x 0.4 cm. The perfusion fluid was a physiological buffer

described by Sickel (1972). The composition of the medium (m moles/l) was: NaCl, 80.0; KCl, 2.0; $CaCl_2$, 0.1; $MgCl_2$, 0.1; NaH_2PO_4, 1.5; Na_2HPO_4, 13.5; glucose, 5.0. The medium was oxygenated with room air using a fish tank air pump. The pH of the medium was 7.4 $\pm$ 0.1 and adjustments in pH were made with NaOH or HCl when necessary. The control and the drug containing media were placed in glass reservoirs positioned above the preparation. These perfusing solutions were gravity fed to a common inlet which permitted regulation of the perfusion rate. The tissue compartment was perfused at a rate of 4 - 6 ml/minute. All preparations were maintained at room temperature (19 - 22° C).

Light stimulation

The light source used for the test flash stimulus was an incandescent lamp operated from a 6.0 V battery. The light was focussed through the optics of an AO Spencer dissecting microscope, providing full field illumination of the retina. In some preparations the test stimulus was reduced to a 1.0 mm light spot by placing a light stop between the lamp and the preparation. The intensity of the light stimulus was varied with neutral density filters (Balzers) over a range of 7 log units. The intensity of the unattenuated light source was 13.35 mW/cm^2, as measured with a radiant flux meter (Hewlett - Packard Model 833 OA). The measured

intensities at various log unit attenuations are given in Appendix A. The wave length of the test stimulus was varied with broad band optical filters. The transmission characteristics of these filters are given in Appendix A. The duration of the stimulus flash was controlled by an electromagnetic shutter (Uniblitz, Model 300C). The stimulus duration was 200 msec for most experiments. This period of stimulation was chosen because with stimulus durations shorter than approximately 150 msec the retinal response becomes a complex function of duration and intensity, while above this critical time, response is related only to luminence intensity (Muller - Limmroth, 1953; Alpern and Faris, 1956). Background light was provided by a 6.0 V incandescent lamp focussed on a reflecting hemisphere surrounding the preparation. This provided diffuse illumination for the entire retina. The intensity of the background light was varied with neutral density filters. The initiation of background light was 4.5 seconds before the presentation of the test stimulus. This has been shown to be sufficient time to allow for change in sensitivity associated with weak background light (Copenhagen, 1975). The duration of background light upon which the test flash was superimposed was 5.0 seconds. The background illumination and test flash were presented every 60 seconds. Easter (1968) reported that in isolated

goldfish retina this procedure would reduce visual pigment bleaching to a minimum. In those preparations in which the retina was bleached *in vitro*, the test stimulus source served as the bleaching light. The duration and intensity of this light source were controlled as outlined above. In the group of animals which was light-adapted prior to sacrifice, the frogs were maintained in clear plastic containers under normal laboratory light conditions (130 fc) for 2 hours. After isolation, the retinas from these animals were placed in the perfusion chamber and tested in the same manner as the dark-adapted animals. The perfusion chamber and recording instruments were housed in a darkened screen room, where instrument lights were covered to limit stray light, and the preparation was tested under scotopic conditions.

Recording electrodes

Gross potential changes were recorded using platinum-iridium electrodes, having a 250 μ diameter and a Teflon coating for insulation. Differential recordings were made with the G1 electrode placed on the receptor surface of the retina and G2 placed directly beneath the retina. The ground electrode was placed in the perfusing media. The gross potential signals were led into a Grass P9 AC pre-amplifier with the band pass filter set at 0.2 - 8,000 Hz. The single-ended output from this amplifier was led into a Hewlett -

Packard storage oscilloscope (Model 141B). Signals displayed on the oscilloscope were recorded on moving film with a Grass model C-4 kymograph camera. The records were later quantified by eye. In some experiments involving the e-wave, the gross potential signals were led into a Grass low level d.c. pre-amplifier (Model 7P1 A) and recorded on a Grass model 7 polygraph. A block diagram of this recording set-up is given in Fig. 1.

Recording of unit potentials was accomplished with platinum-iridium microelectrodes. These electrodes were described by Wagner et al. (1960). Platinum-iridium wire (Medwire Corp.), with a diameter of 250 μ, was electropolished in a 50% NaCN solution made up with 30% NaOH. The electrolyzing current was provided by an a.c. voltage regulator connected between the wire and a carbon rod. Initial shaping of the tip was accomplished with a 5 - 6 V a.c. source. The final polishing, leaving tapered electrodes with tips of 3 - 5 microns, was accomplished at lower voltages (1.0 V a.c.). The electrode was then insulated with Insl - X 33 enamel. This was accomplished by pushing the electrode through a drop of Insl - X supported on a wire hook. The thin layer of enamel was allowed to dry overnight. Two additional coats of enamel were applied in the manner just described. Immediately before using, the tip of the

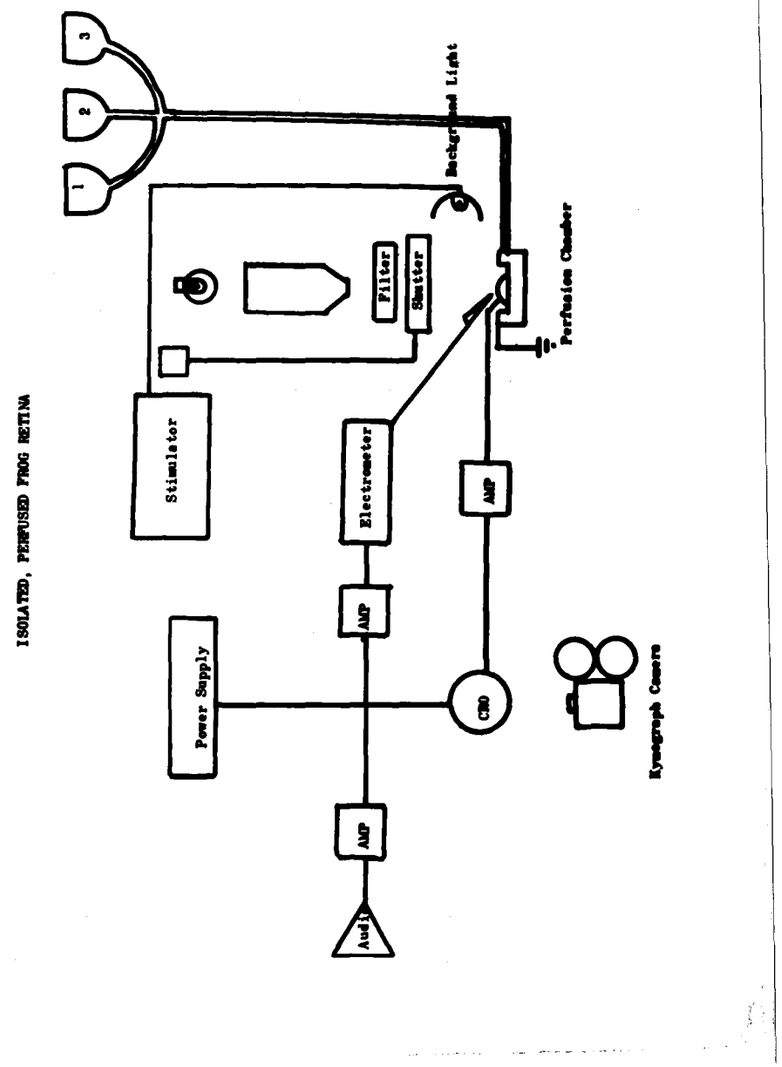


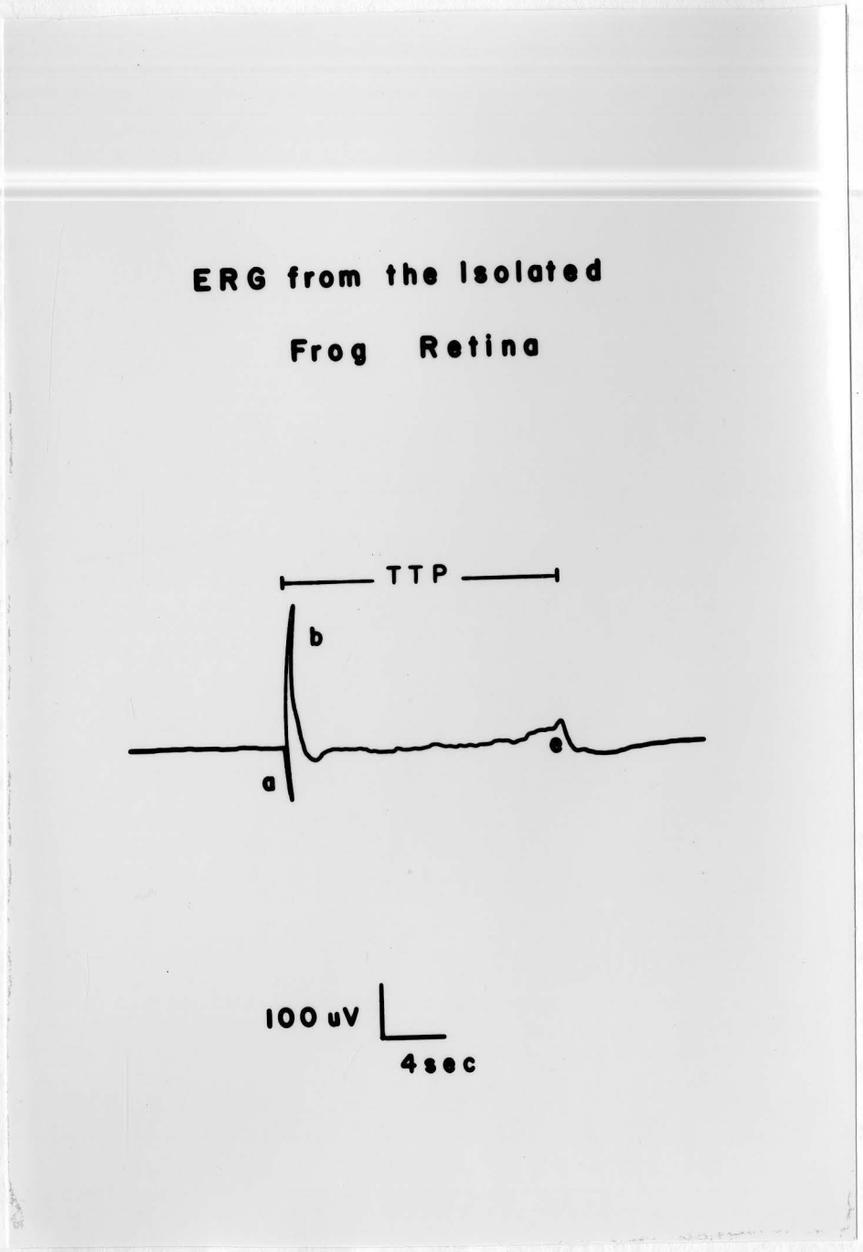
Fig. 1. Diagram of recording set up for isolated, perfused frog retina. The retina was mounted receptor side up in the perfusion chamber and was superfused with physiological buffer. The light source was an incandescent lamp whose intensity was regulated with neutral density filters. Potential changes were recorded using platinum-iridium electrodes. Amplified signals were displayed on an oscilloscope and recorded on film.

electrode was platenized using a 2% platinum chloride solution. This was accomplished by passing current for approximately 30 seconds from a 15 V d.c. source in series with a 1 megohm resistor between the electrode and a platinum-iridium wire immersed in the platinum chloride solution. The electrode was mounted in a Narishige micromanipulator. Unit activity was recorded from the vitreous side of the retina. Signals from the electrode were led into a WPI FET (Model M701). The 1X output was then led into a Tektronix low-level pre-amplifier (Model 122) and finally into a Hewlett - Packard storage oscilloscope (Model 141B) and an audio monitor. Signals displayed on the oscilloscope were recorded on film as described above. Quantification of responses was done by eye. A block diagram of this recording set-up is shown in Fig. 1.

Electrophysiological responses

The retina can, in general, be divided into three layers: the photoreceptor layer, the inner nuclear layer, and the ganglion cell layer. Neuronal activity occurring at each of these layers can be monitored by measuring either gross potential changes or single cell activity. The electroretinogram is the mass potential response associated with light stimulation of the retina. It has a characteristic waveform (Fig. 2) which, in the isolated retina preparation,

Fig. 2. Electroretinogram evoked from the isolated frog retina. a-wave, b-wave and e-wave are designated by the letters a, b and e respectively. TTP refers to the time - to - peak of the e-wave.



is composed of a-, b- and d-waves. Under certain conditions, a delayed off-response, or e-wave, is also observed. The b-wave of the ERG was used in this study as a measure of neuronal activity occurring in the inner nuclear layer. Amplitude and time-course of the b-wave and other components of the ERG were quantified according to the method described by Krill (1972). The a-wave amplitude is measured from the baseline to the lowest point on this wave. The b-wave amplitude is measured from the trough of the a-wave to the highest point of the b-wave. The implicit, or peak, time for each component is the interval from the beginning of the stimulus to the highest point of the wave. The amplitude of the e-wave is measured from the baseline to the highest point of the wave.

In quantification of the electroretinogram, the effect of the b-wave on the a-wave must be considered. The rising edge of the b-wave interrupts the complete development of the a-wave, making evaluation of this response difficult. In order to assess photoreceptor function, therefore, the mass receptor potential was isolated according to the method of Sillman et al. (1969). Treatment of the retina with sodium aspartate suppresses PII and proximal PIII components of the ERG (Sillman et al., 1969). The remaining potential has been shown to consist almost exclusively of distal PIII,

or the receptor response, and is a good measure of activity occurring at the level of the photoreceptors (Normann and Werblin, 1974). In these studies 20 mM sodium aspartate was substituted for 20 mM NaCl in the perfusion media. Perfusion of the retina with sodium aspartate was continued for the duration of the experiment in order to prevent recovery of the PII and proximal PIII components. The amplitude of the aspartate-isolated mass receptor potential was measured from the baseline to the lowest point of the wave. Implicit time for this response indicates the interval between the beginning of the stimulus and the lowest point of the wave.

Neuronal activity occurring in the ganglion cell layer was measured by recording unit activity with extracellular microelectrodes. This was necessary because the ganglion cells contribute little to gross potential responses (Sickel, 1972) and, therefore, cannot be monitored with the ERG. Ganglion cell activity was recorded as spikes/second. Units were isolated using both audio monitoring and visualization of spike activity on the oscilloscope screen.

Measurement of light adaptation

Adaptation is that property of the visual system which allows the eye to process light information under widely changing levels of illumination. Retinal sensitivity at any given level of ambient light could be determined by

measuring absolute threshold. Sensitivity, however, also is a measure of the eye's ability to discriminate small intensity differences. This depends on the steepness of the response vs stimulus intensity function (R - log I), where R is stimulus response and I is stimulus intensity. At any given level of background light this linear portion of the R - log I curve is generally narrow, extending 1 to 3 log units, and comprises the "working range" of the eye. In order to accommodate to changing ambient illumination, the "working range" of the eye must be shifted along the log I scale, and this process has been observed to be fundamental to visual adaptation (Byzov and Kusnezova, 1971). It is changes in the R - log I curves which will be used to follow light-adaptation in the present experiments.

A typical experiment proceeds in the following manner. After placing the retina in the perfusion chamber, the preparation is allowed to equilibrate for 20 minutes in the dark. At this time the R - log I curve for control conditions is generated by stimulating the retina at various intensities once every 60 seconds. The stimulus is alternately presented both in the dark and concomitant with a background light. Progressing from low stimulus intensity to high, from high to low, or randomizing presentation of stimuli of each intensity has no observable effect on the generation of R -

log I curves. After control responses are recorded, the preparation is perfused with the test agent. The preparation is continuously monitored during administration of the test agent. After equilibration (approximately 5 - 10 minutes), the R - log I curve is again generated in the manner described above. The preparation is then perfused with control medium. The preparation is continuously monitored during the "wash out" of the test agent. A R - log I curve is again generated in the control medium in order to observe whether the drug effect when present is reversible. The stimulus intensities generally ranged from threshold to saturation of the response, i.e. where increases in stimulus intensity no longer lead to increases in response. A typical experiment lasts approximately 2 - 2.5 hours.

Drugs

The following drugs were used in these experiments: γ-amino-m-butyric acid (Sigma), picrotoxin (Sigma), bicuculline (Regis), glycine (Sigma), strychnine sulfate (Merk), dopamine HCl (Sigma) and sodium aspartate (Eastman Kodak). All drugs were dissolved in Sickel's media. The final pH was 7.4 ± 0.1 for all drugs except bicuculline, which was 7.2 ± 0.1. This was found to be necessary in order to keep the drug in solution at a concentration of 2×10^{-4} M. In experiments involving bicuculline, the control medium was

adjusted to a pH of 7.2 ± 0.1. The concentration of drugs reported in most experiments is the lowest concentration which produces a significant effect. The change in osmolality brought about by the addition of drug to the perfusion media was not considered significant at the concentrations reported and was not compensated for in the control medium.

The following drugs were surveyed for their effects on the gross potential responses of the retina: acetylcholine, physostigmine, norepinephrine, haloperidol, taurine, histamine, histidine, cyclic 3'5' - AMP, cyclic 3'5' - GMP and phenobarbital.

Except in those experiments in which a neurotransmitter antagonist was used to block the actions of a specific agonist, no retina was exposed to more than one drug. Approximately 200 isolated retina preparations, therefore, were used for this study. The data in each experiment were obtained from retinal preparations used only for that experiment.

Statistical analysis

The experiments were designed so that each animal served as its own control. Accordingly, the paired Student's t-test was used for statistical analysis of the results. The t value was determined by a computer program for paired t-test. The p value was determined from the Significance

Limits of the Student Distribution. A p-value less than 0.05 indicated a statistically significant difference; a p-value greater than 0.05 indicated statistical significance was not proven.

RESULTS

Effect of stimulus intensity on gross potential responses

The aspartate - isolated mass receptor response (AIMRR), elicited by light of increasing intensity, provides a "working range" of approximately 3 log units; that is, measurable changes in response occurred only when the stimulus intensity was less than a thousand-fold above threshold. Fig. 3 shows the response of a representative retina to stimulus intensities increasing in log unit steps (0.0 log I = unattenuated light source). Response amplitudes increase and implicit times diminish with increasing stimulus intensity. Fig. 4(A) illustrates the effect of increasing stimulus intensity on the b-wave of the ERG from a representative preparation. B-wave amplitude increases and implicit time decreases as the stimulus intensity is raised. The "working range" of the b-wave is approximately 3 log units.

Effect of background light on gross potential responses

Background light has been shown to shift R - log I curves to the right and to depress the ceiling response to light stimulation. Fig. 5 indicates the effect of background light at two intensities on the R - log I curve for the aspartate - isolated mass receptor response. The ordi-

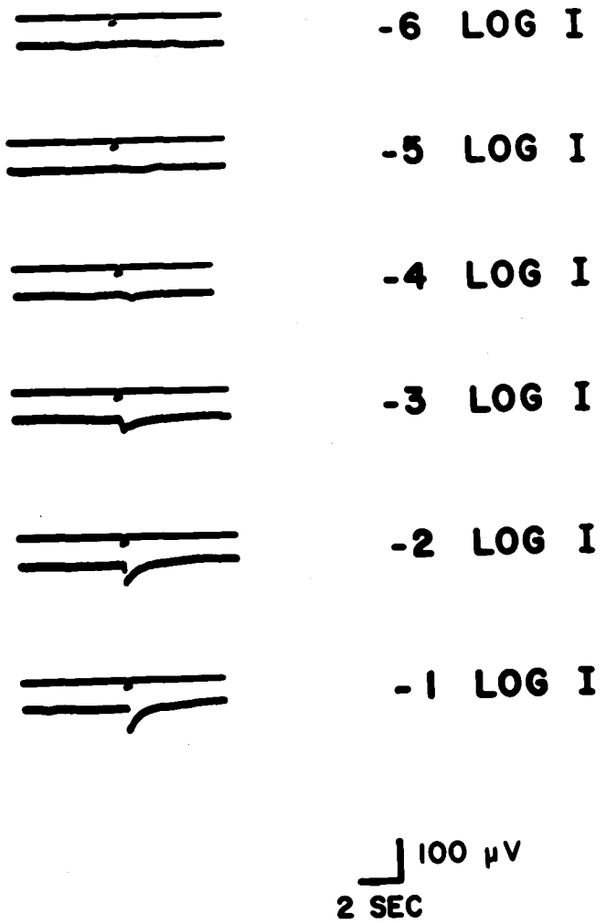


Fig. 3. Aspartate - isolated mass receptor responses to light stimuli of increasing intensity. Log I refers to the log attenuation of the test flash (200 msec); log I = 0.0 = 13.35 mW/cm^2. The line above each tracing indicates the duration of the stimulus. Note that the threshold for this response at -5.0 log I corresponds to the appearance of the a-wave of the ERG (Fig. 4A).

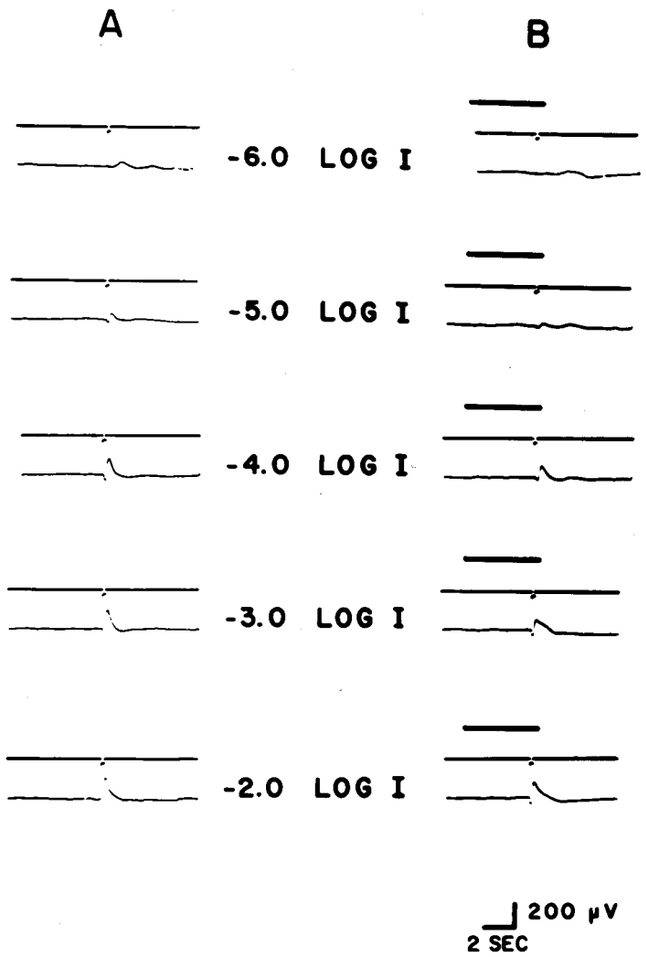


Fig. 4. Effect of background light (0.008 fc) on isolated retina electroretinograms. (A) ERG responses to light stimuli of increasing intensity obtained in darkness. (B) ERG responses to light stimuli of increasing intensity superimposed on a background light (0.008 fc). Log I refers to the log attenuation of the test flash (200 msec). ⟝⟞ represents the 5 second duration of background light. Onset of the flash is indicated by the line above each tracing. Note reduction in ERG amplitude in the presence of background light.

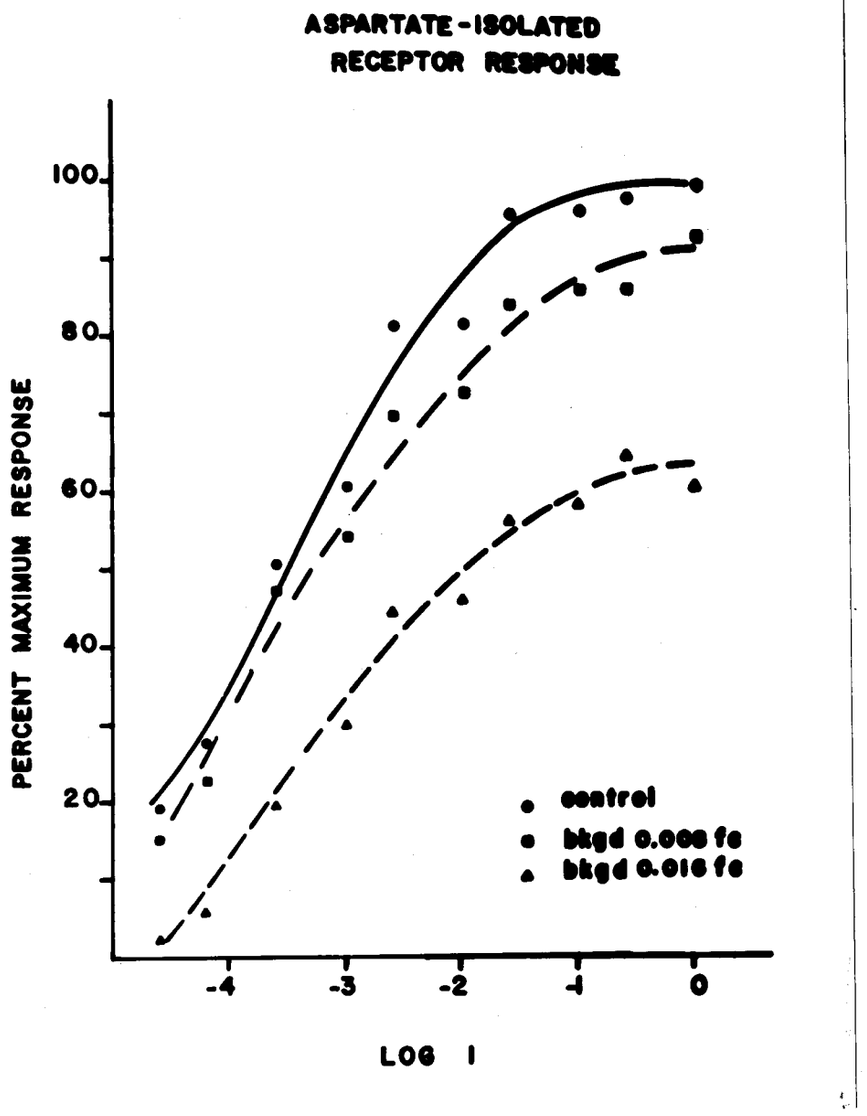


Fig. 5. Effect of background light on the aspartate - isolated mass receptor response R - log I function. The ordinate indicates the per cent maximum response obtained in the dark (control), while the abscissa represents stimulus intensity as log attenuation of the test flash (duration 200 msec) produced by neutral density filters. The radiant flux of the unattenuated test flash at log I = 0.0 was 13.35 mW/ cm^2. Solid circles ● represent responses to light stimuli in the dark. Solid squares ■ represent responses to light stimuli superimposed on a background light of 0.008 fc. Solid triangles ▲ represent responses to light stimuli superimposed on a background light of 0.016 fc. n = 4. Range of S.E. for data points is ± 0.4 to 9.2%.

nate indicates the response amplitude as per cent of the maximum response obtainable in the dark. The abscissa gives the stimulus intensity in terms of log attenuation of the 13.35 mW/cm^2 light source. Under dark-adapted conditions the R - log I curve shows a "working range" of approximately 3 log units. When the test stimulus is superimposed on a background light of 0.008 fc, no significant change in the response occurs. However, when the test stimulus is superimposed on a background light of 0.016 fc, the R - log I curve shift to the right of approximately 1 log unit is statistically significant and the maximum obtainable response is decreased about 40%. These results are summarized in Table 1 - Appendix B.

The effect of background light of 0.008 fc on the R - log I curve of the b-wave was examined to determine whether or not this weak background light could influence retinal activity proximal to the photoreceptors. Fig. 4 depicts ERG responses from a representative experiment over a stimulus - intensity range of -7.0 to -2.0 log units in the dark and in the presence of background light. The b-wave amplitude is reduced when the stimulus evoking this response is superimposed on background illumination. It is clearly shown at -5.0 log units. Fig. 6 demonstrates that this level of background light causes a shift of the b-wave R -

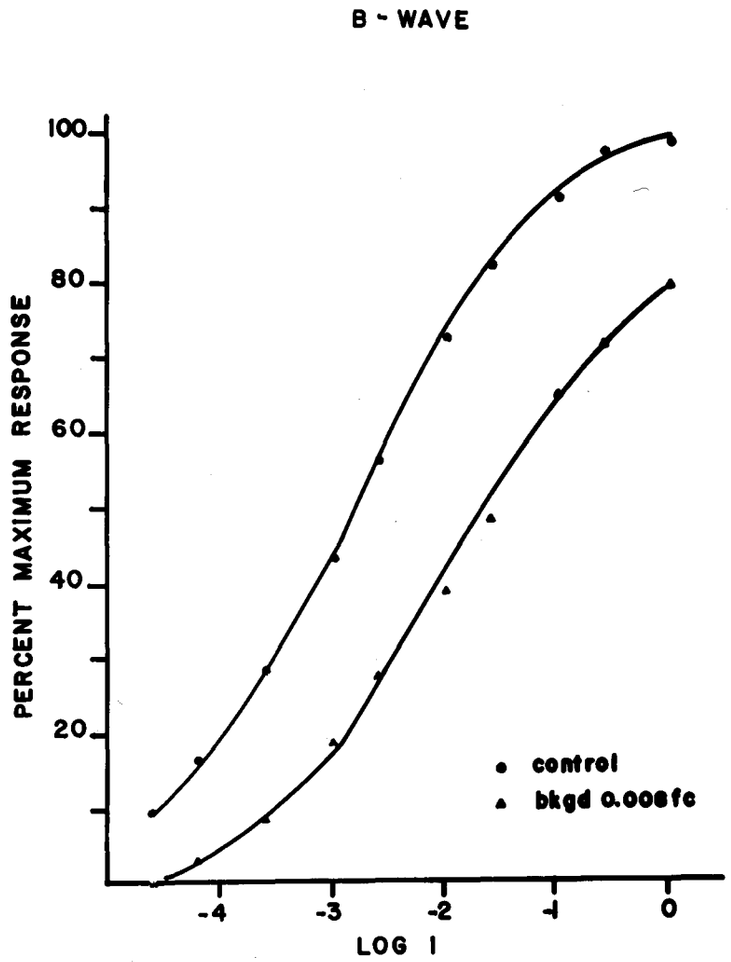


Fig. 6. Effect of background light on the R - log I function of the b-wave of the ERG. Ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark. Solid triangles ▲ represent responses to light stimuli superimposed on a background light of 0.008 fc. Note that the maximum response obtained under these conditions is approximately 80% of the control response. n = 6. Range of S.E. for data points in ±1.2 to 4.9%.

log I curve approximately 1 log unit to the right and depresses the maximum obtainable response about 20%. These results are summarized in Table 2 - Appendix B.

The influence of background light on retinal activity can be demonstrated by changes in implicit times as well as by alterations of the amplitude of the gross potential responses. Fig. 7 shows the effect of background light (0.008 fc) on the implicit time of the aspartate - isolated mass receptor response. Under control conditions the implicit time is inversely related to stimulus intensity, having an average duration of 150 $\pm$ 12.9 msec at 0.0 log I and 675 $\pm$ 47.9 msec at -4.2 log I. A suggestion of a "kink" appears at -2.0 log units. Its possible physiological significance will be discussed later. The presence of background light produces a statistically significant decrease in implicit time over the range of intensities from -4.6 to -2.0 log units. The per cent reduction in implicit time ranges from 15% at -2.0 log I to 26% at -4.6 log I (Table 3 - Appendix B). With a background light of 0.016 fc, the implicit time is significantly decreased from control at stimulus intensities from -4.6 log units to -3.0 log units (Fig. 8). The per cent reduction in implicit time ranges from 23.5% at -3.0 log I to 56.8% at -4.6 log I (Table 3 - Appendix B).

Fig. 9 illustrates the effect of background light

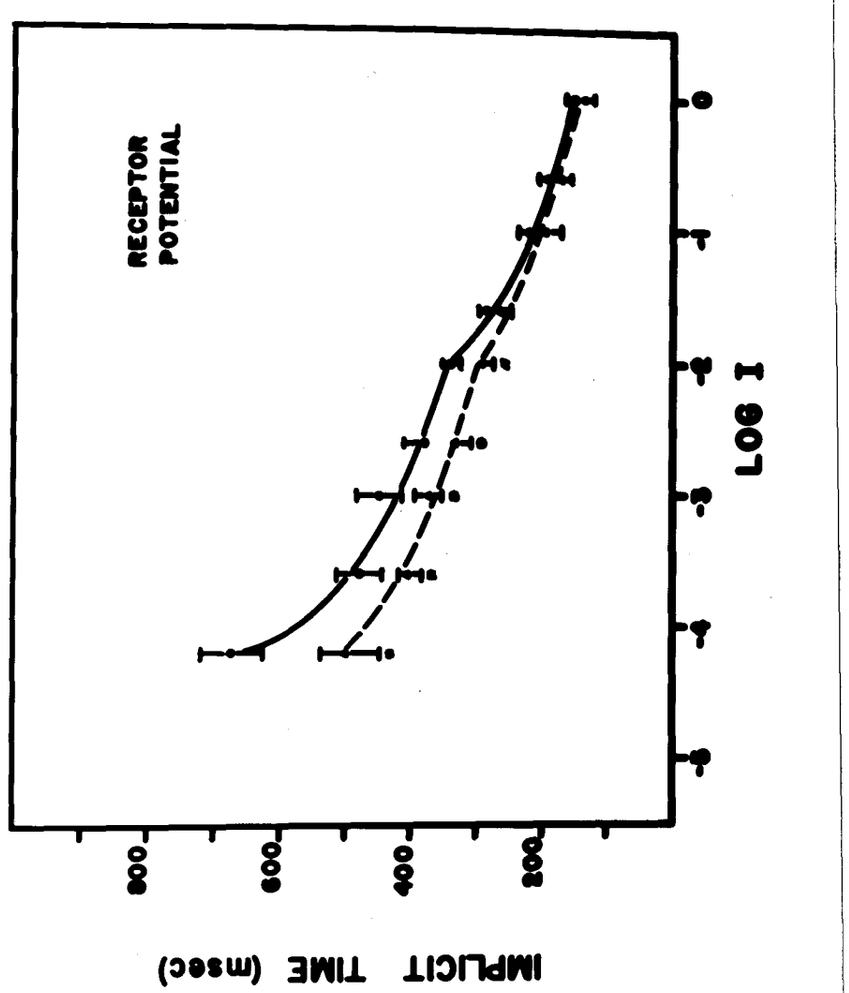


Fig. 7. Effect of background light on the implicit time of the aspartate - isolated mass receptor response. The ordinate represents the implicit time in msec. The abscissa gives stimulus intensity as log attenuation of the test flash (200 msec) produced by neutral density filters. Solid circles ●—● represent receptor response implicit times obtained with light stimuli in the dark. Solid triangles ▲---▲ represent receptor response implicit times obtained with light stimuli superimposed on background light (0.008 fc). Note the suggestion of a break in the curve or "kink" at -2.0 log I stimulus intensity. n = 6. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$).

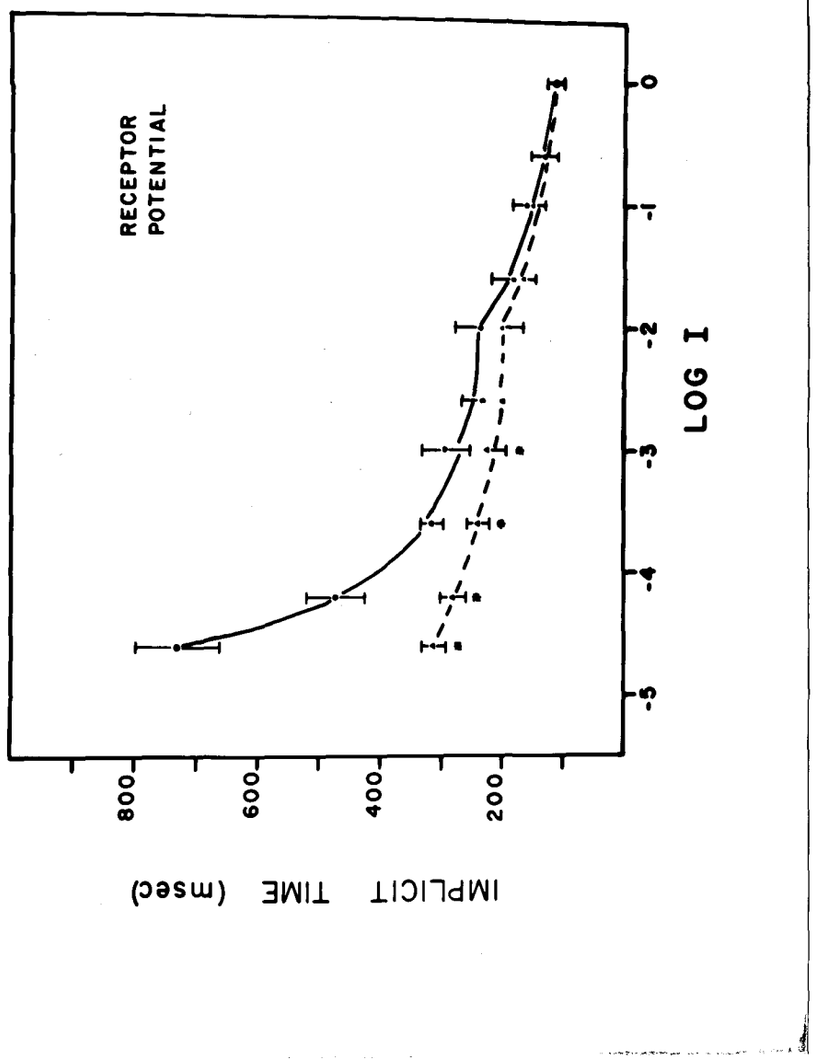


Fig. 8. Effect of background light on the implicit time of the aspartate - isolated mass receptor response. Ordinate and abscissa are as described in Fig. 7. Solid circles ●—● represent receptor response implicit times obtained with light stimuli in the dark. Solid triangles ▲---▲ represent receptor response implicit times obtained with light stimuli superimposed on background light (0.016 fc). n = 4. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$). Note the break in the curve which is especially pronounced in the control group.

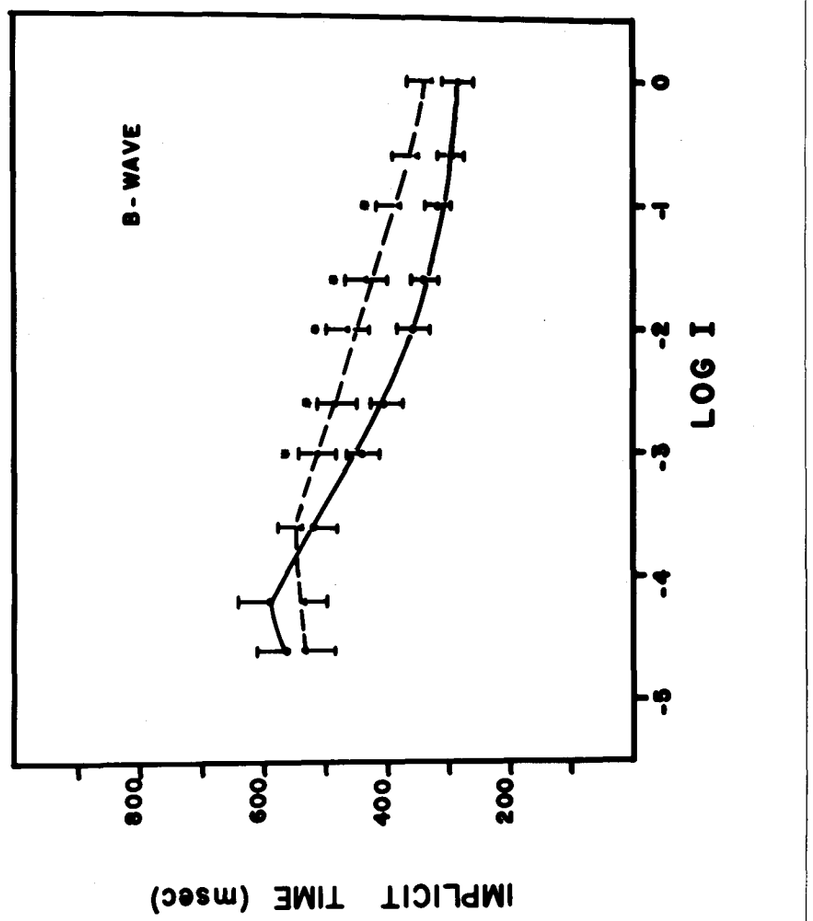


Fig. 9. Effect of background light on the implicit time of the b-wave of the ERG. The ordinate and the abscissa are as described in Fig. 7. Solid circles ●—● represent b-wave implicit time obtained with light stimuli in the dark. Solid triangles ▲---▲ represent b-wave implicit times obtained with light stimuli superimposed on background light (0.008 fc). n = 12. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$). Note background light significantly lengthens b-wave implicit time at stimulus intensities of -3.0 to -1.0 log I.

at 0.008 fc on the implicit time of the b-wave. Under control conditions an inverse relationship between b-wave implicit time and stimulus intensity occurs. In the presence of weak background light (0.008 fc), the reduction of b-wave implicit time with increasing stimulus intensity is significantly less profound at stimulus intensities of -1.0 to -3.0 log I than under control conditions. These results are summarized in Table 4 - Appendix B.

Fig. 10(A) reproduces oscilloscope tracings from a representative experiment in which a change in waveform of the isolated receptor response from biphasic to monophasic takes place at approximately -2.0 log units and which is seen thereafter at all higher light intensities employed. Fig. 10(B) demonstrates that a similar change in waveform of the a-wave of the ERG occurs at approximately -2.0 log I. A "doublet" configuration of the a-wave is elicited. In a plot of implicit time vs stimulus intensity, this point heralds a break in the curve. This break, or "kink", in the implicit time vs stimulus intensity curves for the aspartate - isolated mass receptor response can be interpreted to represent a transition point for a change from rod to cone domination of the response (cf Figs. 7, 8). Fig. 11 illustrates the effect of background light (0.008 fc) on the waveform of the aspartate - isolated mass receptor response. This level

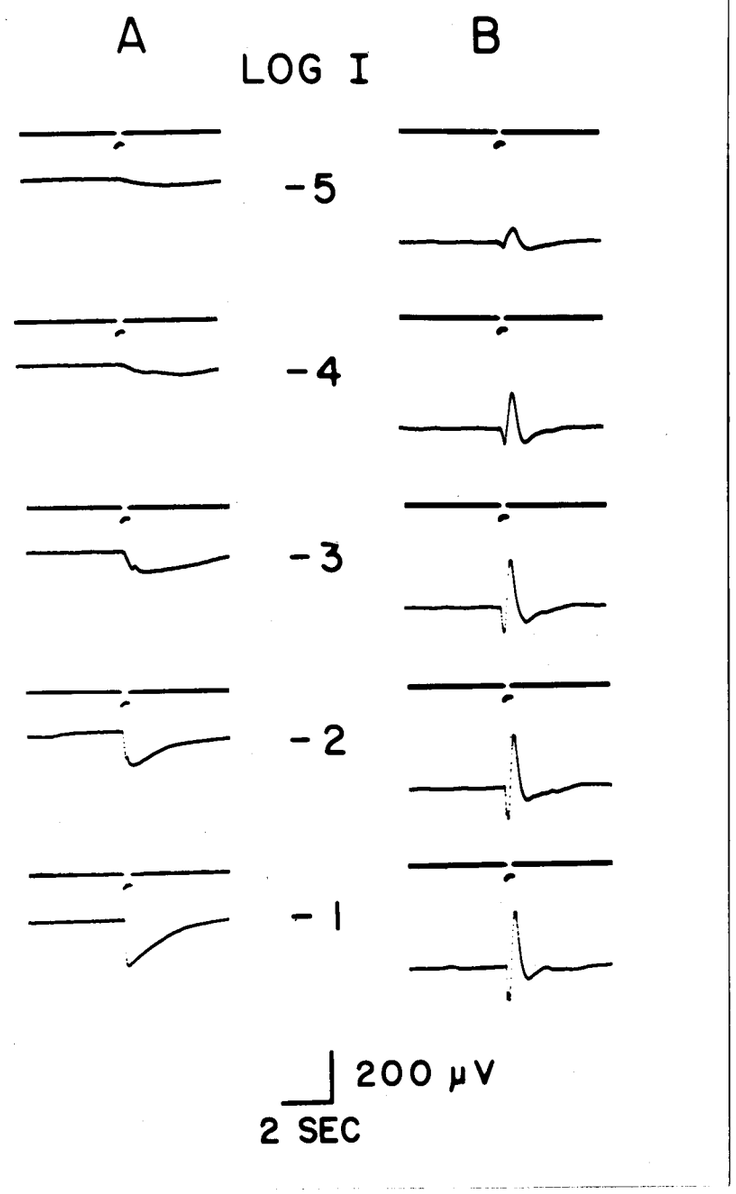


Fig. 10. Waveform changes in the AIMRR and the ERG. (A) aspartate - isolated mass receptor responses to light stimuli of increasing intensity. (B) ERG responses to light stimuli of increasing intensity. Log I refers to the log attenuation of the test flash (200 msec) produced by neutral density filters. -2.0 log I indicates the point of transition from biphasic to monophasic aspartate - isolated mass receptor responses and from monophasic to biphasic a-waves of the ERG. The line above each tracing indicates stimulus duration.

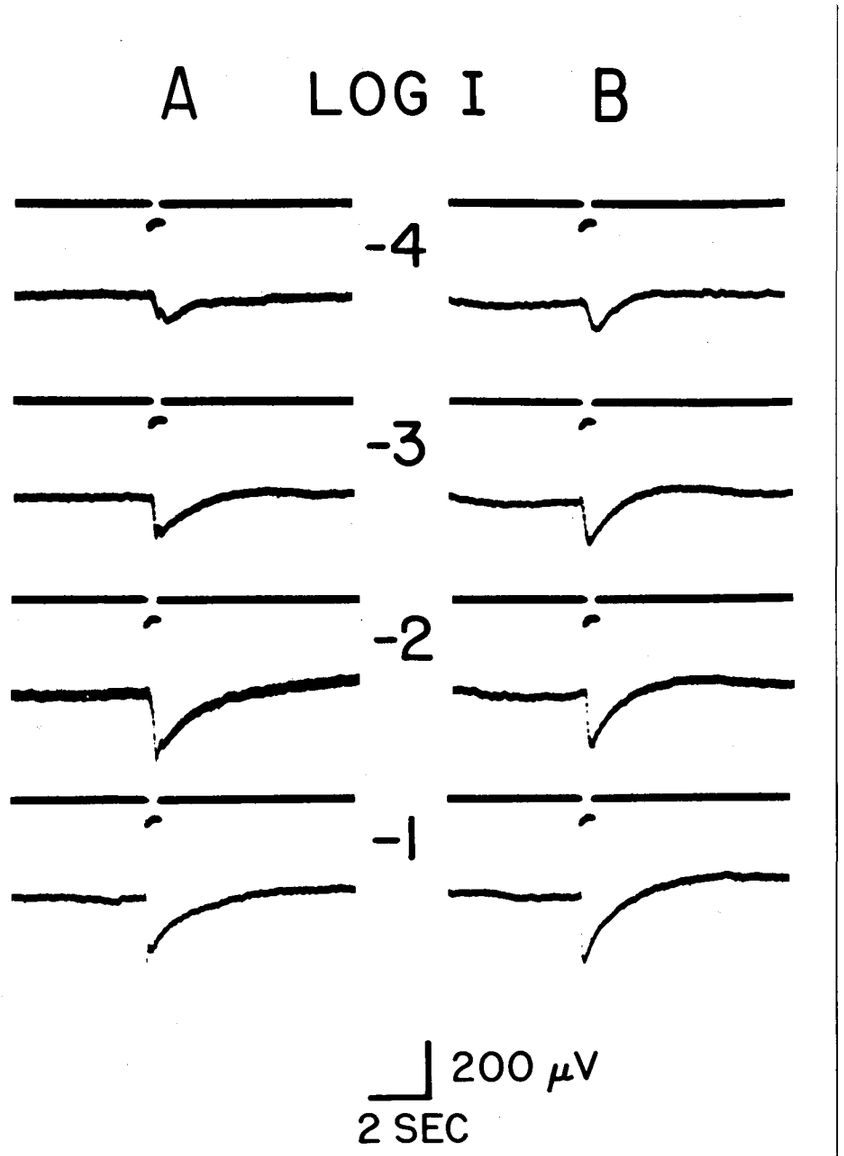


Fig. 11. Suppression of biphasic aspartate - isolated mass receptor responses by background light. (A) potentials elicited by light stimuli of increasing intensity in the dark. (B) potentials elicited by light stimuli of increasing intensity superimposed on background light (0.008 fc). Log I refers to the log attenuation of the test flash (200 msec) produced by neutral density filters. The line above each tracing indicates stimulus duration. Note clear suppression of biphasic waveform at -3.0 and -4.0 log I.

of background light suppresses the biphasic nature of the response seen in Fig. 10. Fig. 12 demonstrates that the "doublet" a-wave of the ERG is also suppressed by background illumination.

GABA and light-adaptation

The effect of GABA, a putative retinal neurotransmitter, on light-adaptation was examined because the shifts of R - log I curves due to weak background light appear to be related to mechanisms operating proximal to the photoreceptors where synaptic interactions can take place. Fig. 13 demonstrates that the addition of GABA to the perfusing medium in concentrations from 10^{-5} to 10^{-3} M causes a reduction in amplitude of the b-wave. The onset of this drug effect was seen approximately 2.5 minutes after beginning of perfusion with media containing GABA (10^{-5} and 10^{-4} M). Recovery of responses occurred approximately 2.5 min after switching to control media. At 10^{-3} M the onset of GABA's action was about 2 minutes, while recovery took approximately 3 min. The isolated retina preparation was routinely perfused with GABA for 5 minutes before eliciting a stimulus - response curve. Similarly, the retina was washed with control media for 5 minutes before recovery responses were recorded. The influence of GABA at 10^{-4} M (the lowest concentration causing a significant effect) on the R - log I curves

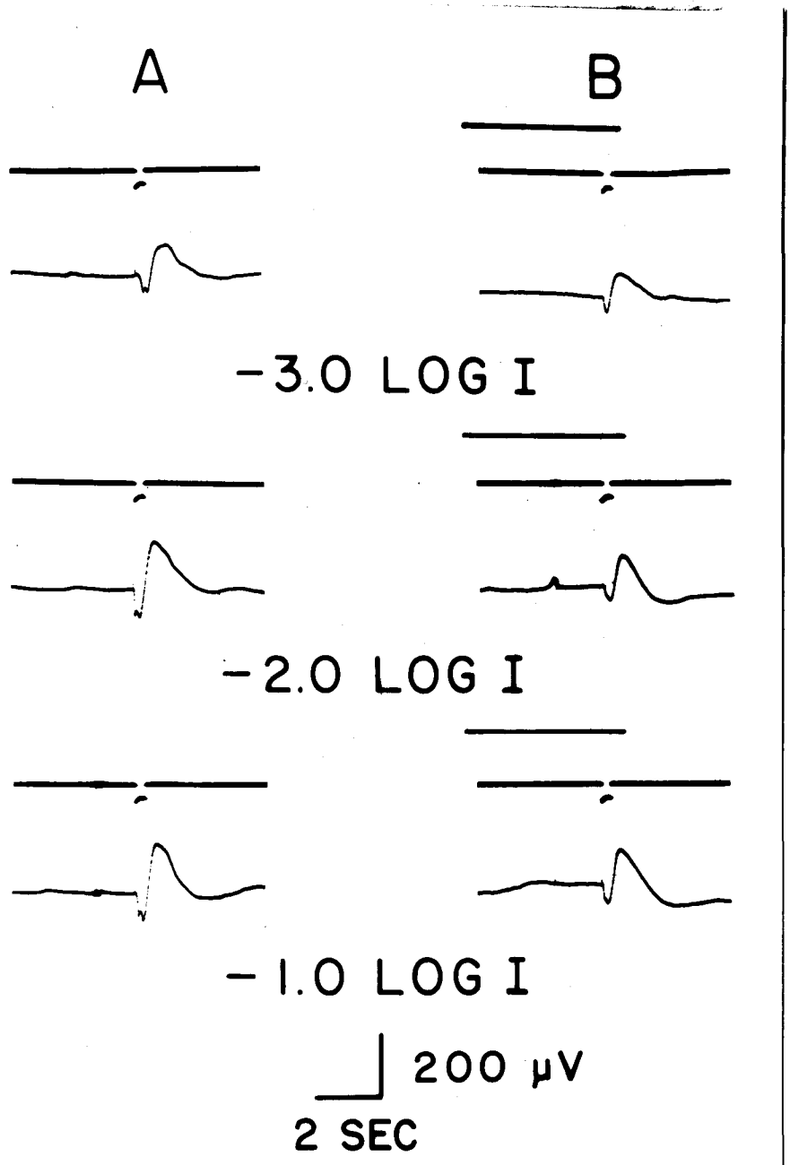


Fig. 12. Suppression of the biphasic a-wave of the ERG by background light. (A) potentials elicited by light stimuli of increasing intensity in the dark. (B) potentials elicited by light stimuli of increasing intensity superimposed on background light (0.008 fc). Log I refers to the log attenuation of the test flash (200 msec) produced by neutral density filters. Note clear suppression of biphasic waveform at -2.0 and -1.0 log I.

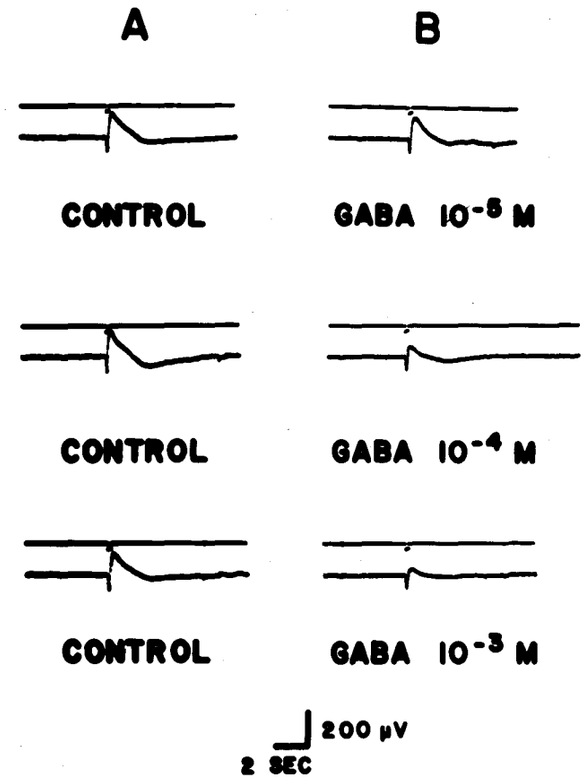


Fig. 13. Effect of GABA on b-wave amplitude. (A) control responses. Line above each tracing indicates the duration of light stimulus. (B) responses after a series of doses of GABA. The onset of response began approximately 2.5 minutes after begining of perfusion with media containing GABA. The effects of the drug were fully reversible with control amplitudes returning after approximately 2.5 minutes of perfusion with control media. Note the decrement in b-wave amplitude is directly proportional to GABA concentration.

in the light and dark is shown in Fig. 14. In the dark, GABA shifts the R - log I curve about 1 log unit to the right and reduces the maximum response obtainable by approximately 30%. The presentation of background light causes a further reduction in b-wave amplitude, augmenting the shift of the R - log I curve to the right about 1.5 log units. Although the overall retinal response is depressed, the changes in retinal sensitivity produced by weak background illumination do not appear to be altered. That is, the shift of the R - log I curve along the log I scale produced by background light is approximately the same both in the presence and absence of drug. These results are summarized in Table 5 - Appendix B.

The b-wave implicit time in these experiments decreased as stimulus intensity increased, ranging from 875 $\pm$ 48 msec at -6.0 log I to 337 $\pm$ 24 msec at -1.0 log I. GABA (10^{-4} M) administration did not significantly alter implicit time (Fig. 15) although it reduced the amplitude of this response.

The influence of GABA on the aspartate - isolated mass receptor response was examined in order to determine whether or not the effect of GABA on retinal responses was due to a direct action on the photoreceptors. GABA (10^{-4} M) has no effect on amplitude of the receptor response over the

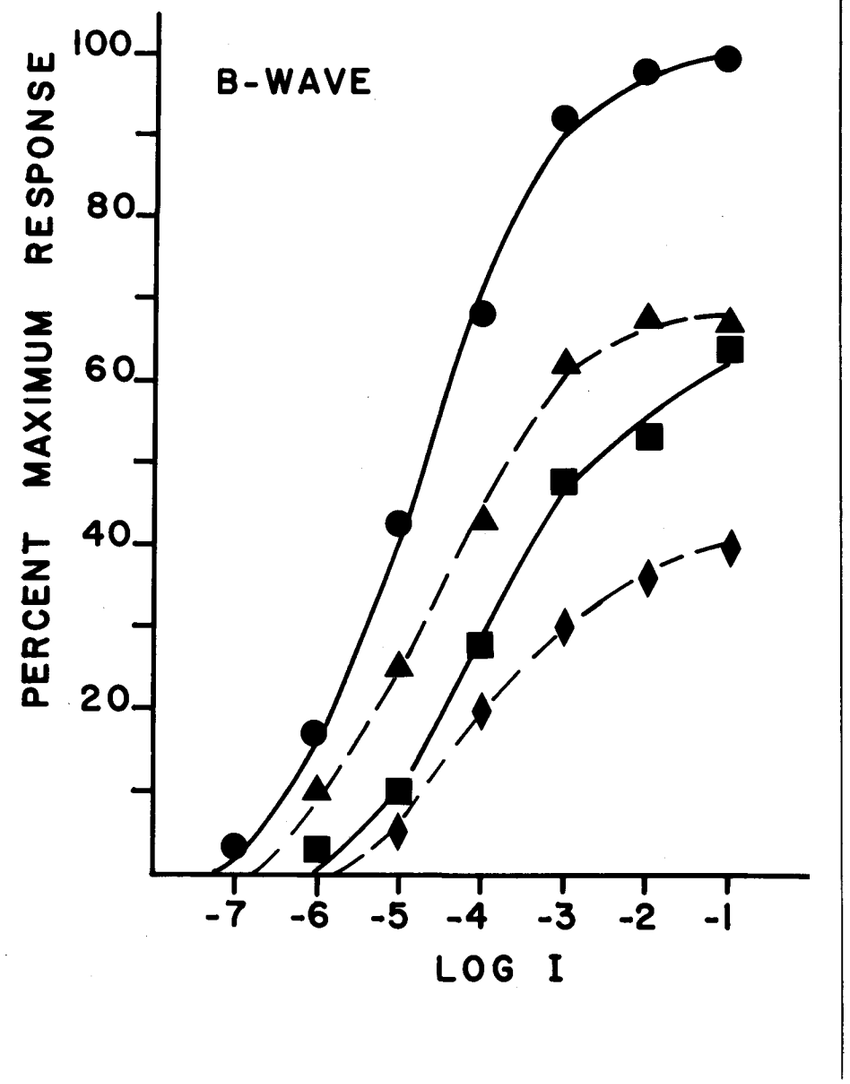


Fig. 14. Effect of GABA on the R - log I functions of the b-wave. Ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for control medium. Solid squares ■ represent responses to light stimuli superimposed on background light (0.008 fc). Solid triangles ▲ represent responses to light stimuli in the dark for medium containing GABA (10^{-4} M). Solid diamonds ◆ represent responses to light stimuli superimposed on background light (0.008 fc) for medium containing GABA (10^{-4} M). n = 7. Range of S.E. for data points is ± 1.3 to 7.1%.

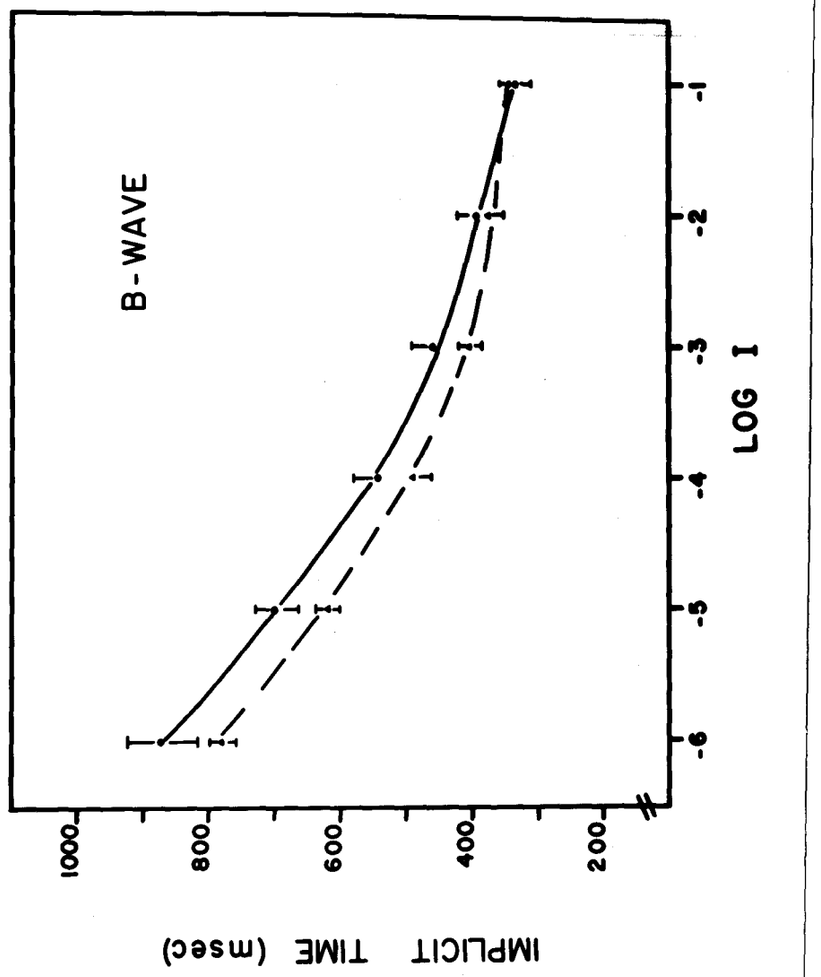


Fig. 15. Effect of GABA on the implicit time of the b-wave. The ordinate and abscissa are as described in Fig. 7. Solid circles •——• represent b-wave implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲----▲ represent b-wave implicit times obtained with light stimuli in the dark with retinas superfused by media containing GABA (10^{-4} M). n = 7. Vertical brackets indicate S.E.M. Note these curves do not differ significantly from each other.

entire range of stimulus intensities examined (Fig. 16; Table 6 - Appendix B). The implicit time of the receptor response is unaltered by GABA (Fig. 17).

Picrotoxin vs GABA

If the observed action of GABA in the retina is mediated by its interaction with GABA receptors, then this action should be reversible by GABA antagonists. Fig. 18 demonstrates this effect of picrotoxin on GABA depression in two representative experiments. Picrotoxin (10^{-5} M) reverses the depression of b-wave amplitude produced by GABA. Picrotoxin (10^{-4} M) not only reverses the GABA depression but augments b-wave amplitude above control levels. The effect of picrotoxin alone was examined to see how it per se might affect the isolated retina ERG. The influence of picrotoxin at 10^{-4} M (the lowest concentration giving a significant effect) on the ERG at three different stimulus intensities is illustrated in Fig. 19. B-wave amplitude at -2.0, -3.0 and -4.2 log I increases 66%, 133% and 100%, respectively, above control. Picrotoxin's influence on the R - log I curves for b-wave amplitude in the dark and in the presence of background light (0.008 fc) are depicted in Fig. 20 (the data are summarized in Table 7 - Appendix B). Picrotoxin (10^{-4} M) increases the b-wave amplitude in the dark 22.6% with unattenuated stimuli and shifts the R - log I curve to the left

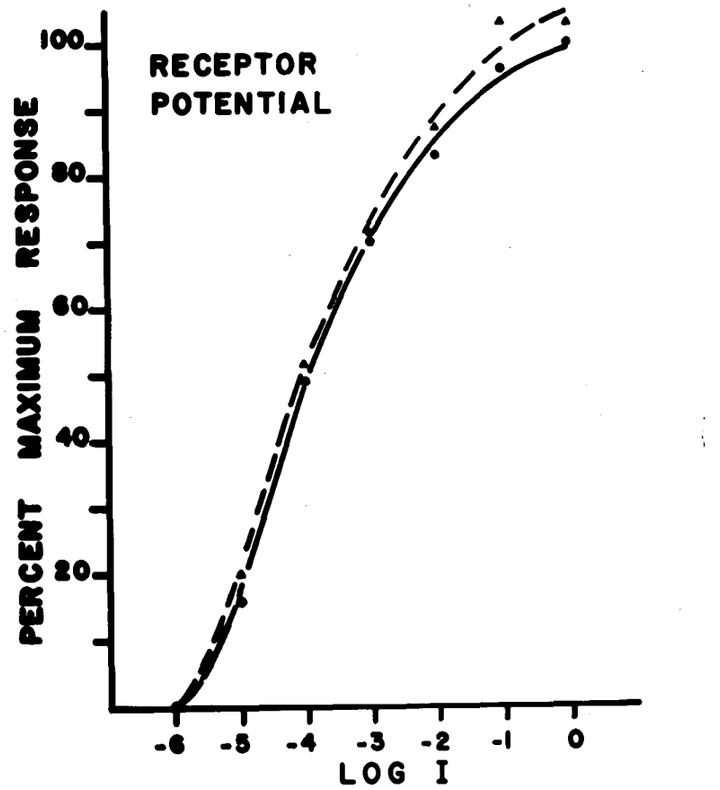


Fig. 16. Effect of GABA on the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 5. Solid circles •——• represent responses to light stimuli in the dark for control medium. Solid triangles ▲- - - -▲ represent responses to light stimuli in the dark for medium containing GABA (10^{-4} M). n = 4. Range of S.E. for data points is ± 1.6 to 5.7%. Responses in the presence of GABA are not significantly different from control responses.

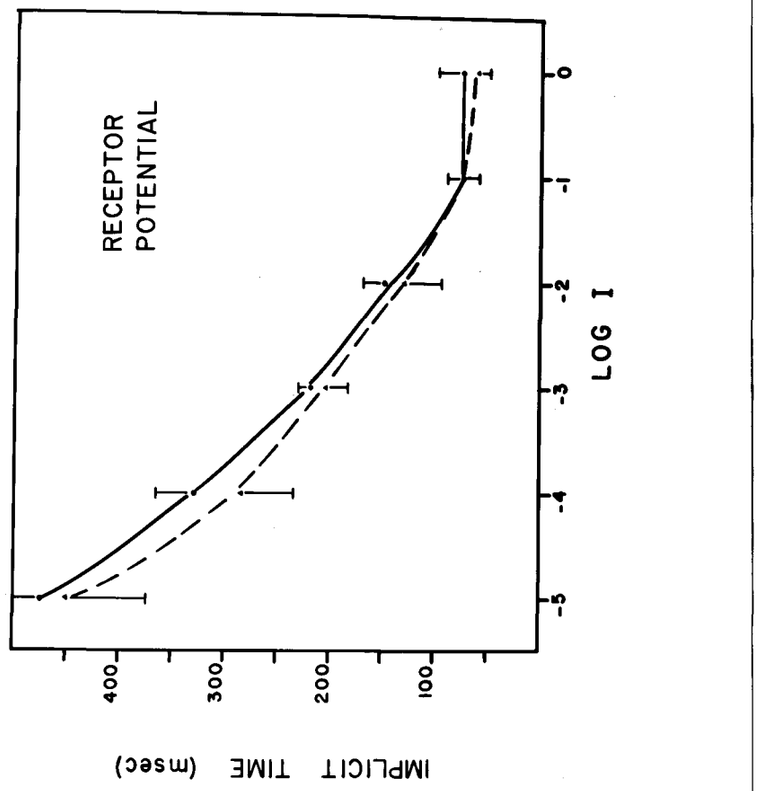
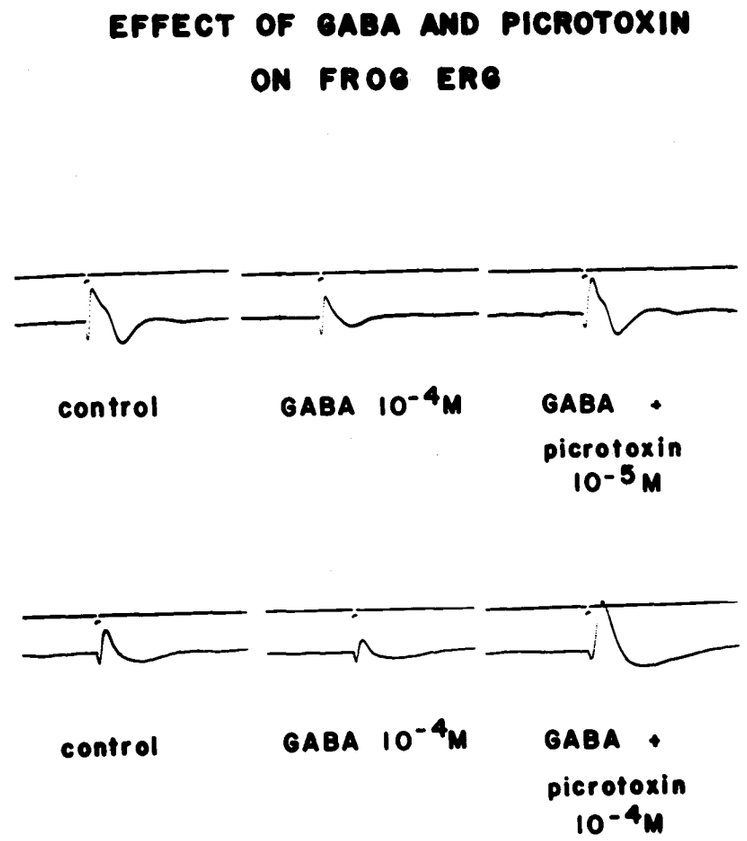


Fig. 17. Effect of GABA on the implicit time of the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 7. Solid circles •——• represent AIMRR implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲----▲ represent AIMRR implicit times obtained with light stimuli in the dark for medium containing GABA (10^{-4} M). n = 4. Vertical brackets indicate S.E.M. Implicit times in the presence of GABA are not significantly different from control.

Fig. 18. Effect of GABA and picrotoxin on the frog ERG. The upper panel shows responses to light stimuli (200 msec; -2.0 log I) with control superfusion medium, with medium containing GABA (10^{-4}M), and with medium containing GABA (10^{-4}M) and picrotoxin (10^{-5}M). The lower panel shows responses to light stimuli (200 msec; -2.0 log I) with control superfusion medium, with medium containing GABA (10^{-4}M), and with medium containing both GABA (10^{-4}M) and picrotoxin (10^{-4}M). Note when equimolar concentrations of picrotoxin and GABA are used the ERG amplitude is greater than control.



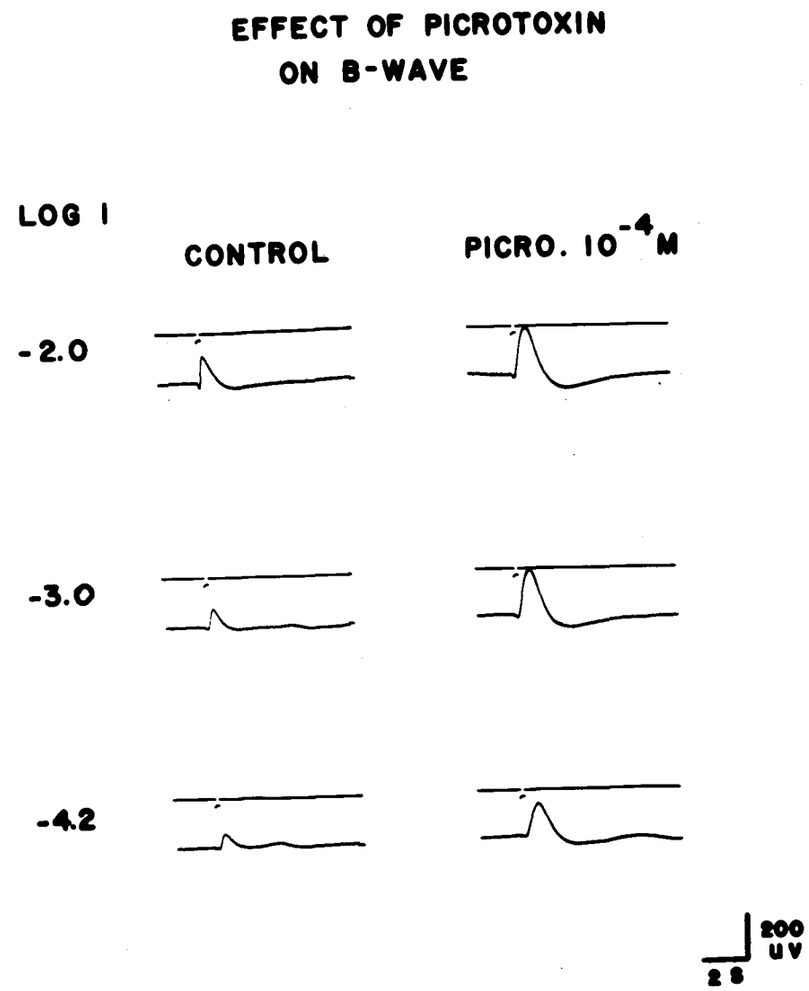


Fig. 19. Effect of picrotoxin on the b-wave of the ERG. The panel on the left reproduces traces of responses to light stimuli at three stimulus intensities for control superfusion medium. The panel on the right illustrates responses to light stimuli at the same three stimulus intensities but where the superfusion medium contains picrotoxin (10^{-4}M). Log I refers to log attenuation of the test flash (200 msec) produced by neutral density filters. Note the large increase in b-wave amplitude and implicit time produced by picrotoxin.

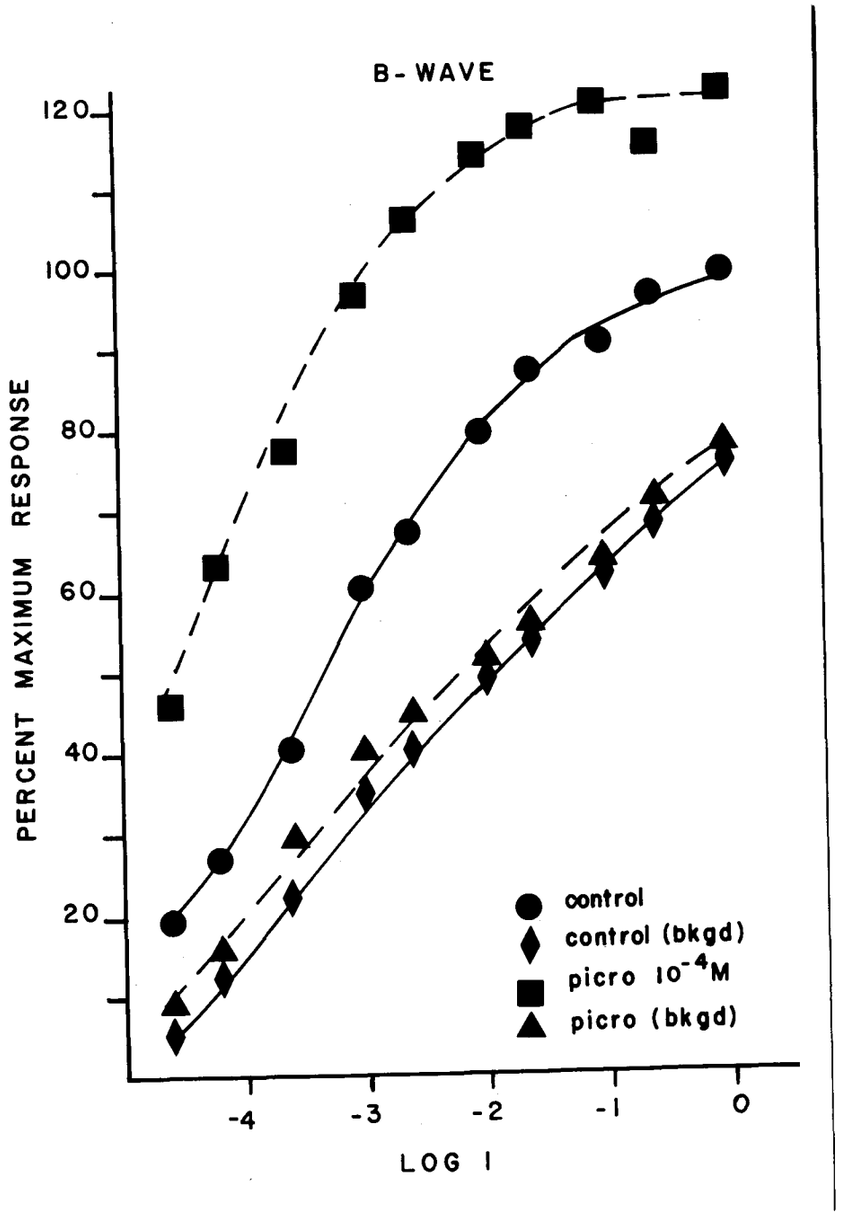


Fig. 20. Effect of picrotoxin on the b-wave R - log I functions. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for the control medium. Solid diamonds ◆ represent responses to light stimuli superimposed on background light (0.008 fc) for the control medium. Solid squares ■ represent responses to light stimuli in the dark for medium containing picrotoxin (10^{-4}M). Solid triangles ▲ represent responses to light stimuli superimposed on background light (0.008 fc) for medium containing picrotoxin (10^{-4}M). n = 6. Range of S.E. for data points is ±1.8 to 16.4%.

approximately 1 log unit. Background light (0.008 fc) with picrotoxin in the bathing medium depresses the maximum response obtainable 36.3% at 0.0 log I and shifts the R - log I curve to the right approximately 2.5 log units, which is greater than that seen in the absence of drug. Picrotoxin essentially doubles the length of b-wave implicit time over the entire range of stimulus intensities studied. The range is from 232% at 0.0 log I to 190% at -4.6 log I (Fig. 21) and differences are statistically significant at each of the 10 measurement points that delimit the curves.

The above studies were performed in animals dark-adapted overnight. The following experiment was performed using a group of animals which were light-adapted two hours prior to isolation of the retina. With these animals the presentation of weak background light (0.008 fc) is without apparent effect on responses. The R - log I curve and the maximum obtainable response are unaltered (Fig. 22). Picrotoxin (10^{-4} M), on the other hand, shifts the R - log I curve to the left approximately 1 log unit and increases the maximum obtainable response by about 30%, just as in dark-adapted animals. In the presence of picrotoxin, presentation of weak background light (0.008 fc) continues to be without effect on these parameters (these results are summarized in Table 8 - Appendix B).

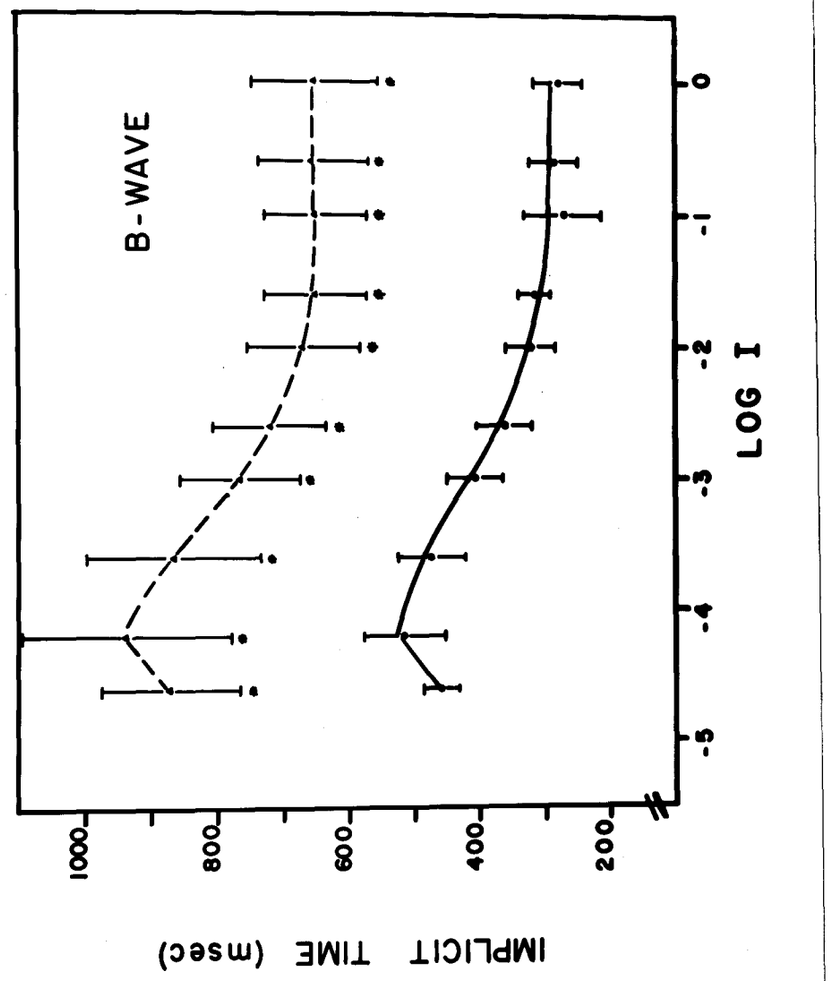


Fig. 21. Effect of picrotoxin on the implicit time of the b-wave. The ordinate and abscissa are as described in Fig. 7. Solid circles •—• represent b-wave implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲---▲ represent b-wave implicit times obtained with light stimuli in the dark for medium containing picrotoxin (10^{-4}M). n = 6. Vertical brackets indicate S.E.M.

* indicates significant difference from control ($p < 0.05$).

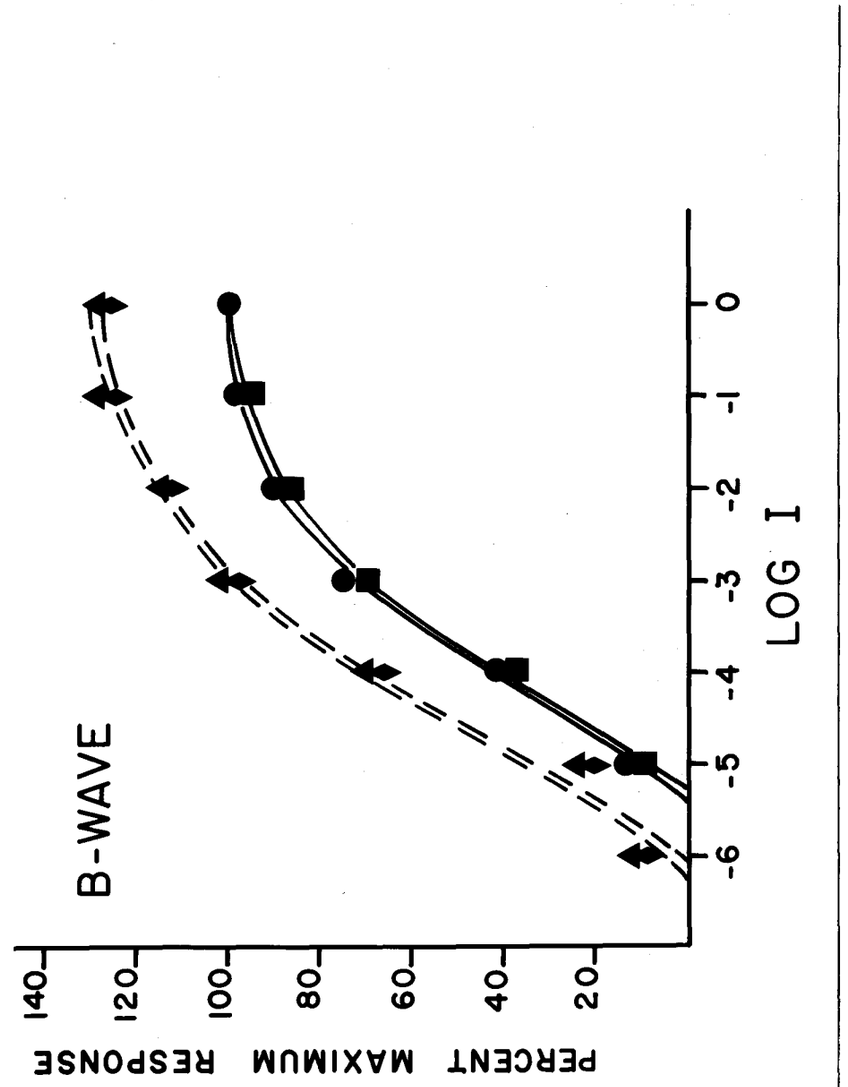


Fig. 22. Effect of picrotoxin on b-wave R - log I functions of retinas isolated from light-adapted animals. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for control medium. Solid squares ■ represent responses to light stimuli superimposed on background light (0.008 fc) in preparations superfused with control medium. Solid triangles ▲ represent responses to light stimuli in the dark for preparations superfused with medium containing picrotoxin (10^{-4}M). Solid diamonds ◆ represent responses to light stimuli superimposed on background light (0.008 fc) for preparations superfused with medium containing picrotoxin (10^{-4}M). n = 3. Range of S.E. for data points in ± 0.4 to 8.3%.

The influence of picrotoxin (10^{-4} M) on the aspartate - isolated mass receptor response was examined in order to determine whether or not the observed effects of picrotoxin were due to a direct action on photoreceptors. Picrotoxin (Fig. 23; Table 9 - Appendix B) had no effect on the amplitude of the mass receptor potential over the entire range of stimulus intensities studied, which would indicate that its primary action is proximal to the photoreceptors. This is further supported by an examination of the implicit time of the receptor potential. Fig. 24 shows that the implicit time of the receptor response is unaffected by exogenous picrotoxin.

Glycine and light adaptation

Glycine has been postulated to be an inhibitory transmitter in the retina because it is a normal constituent of the inner nuclear layer and has an inhibitory action on retinal neurons. Addition of glycine to the perfusion medium produces a dose-dependent depression of the ERG b-wave. This effect is demonstrated in tracings from a representative experiment in which glycine at doses of 10^{-5} to 10^{-3} M was studied (Fig. 25). At concentrations of 10^{-5} to 10^{-4} M the maximum effect of glycine was seen after approximately 3 minutes of perfusion with the drug. Control responses returned in about 1.5 minutes after changing to control medium.

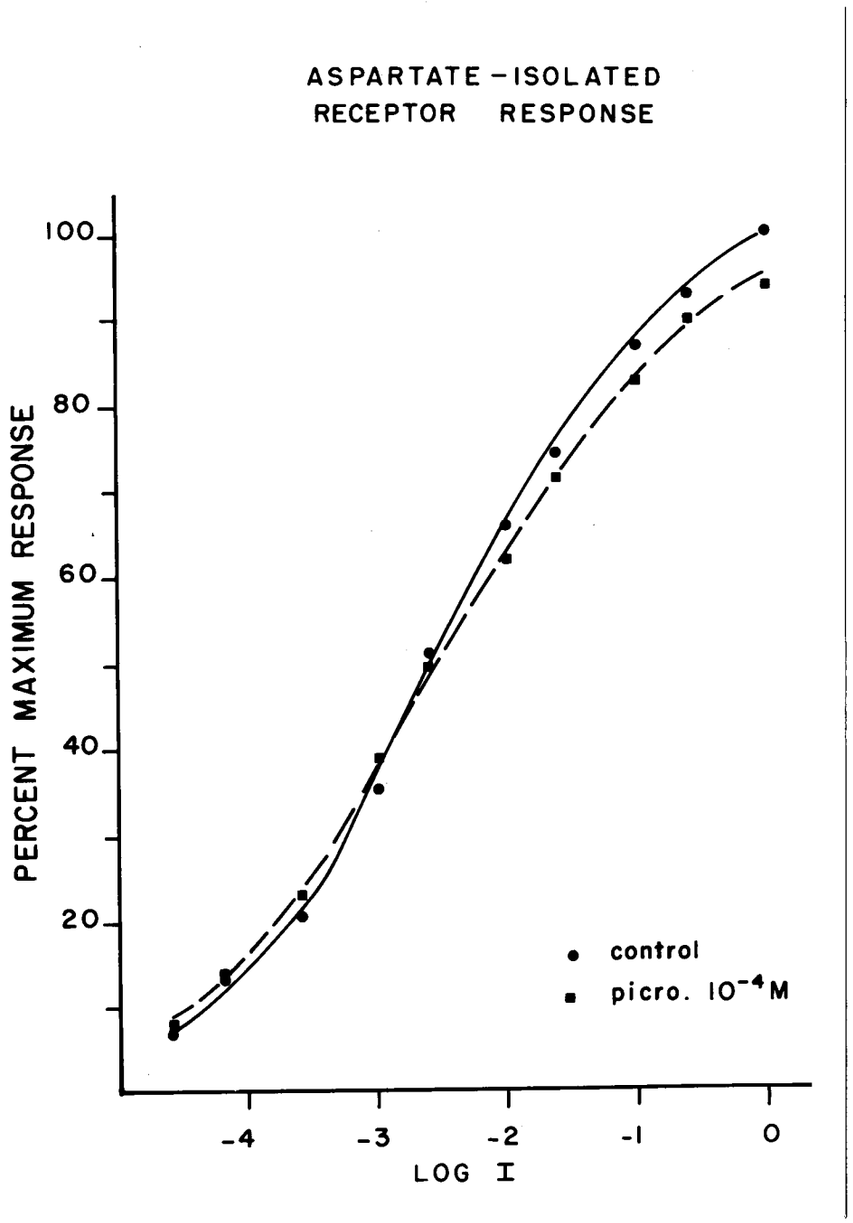


Fig. 23. Effect of picrotoxin on the R - log I function of the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 5. Solid circles •—• represent responses to light stimuli in the dark in preparations superfused with control medium. Solid squares ■----■ represent responses to light stimuli in the dark for preparations superfused with medium containing picrotoxin (10^{-4}M). n = 6. Range of S.E. for the data points in ±2.1 to 4.7%.

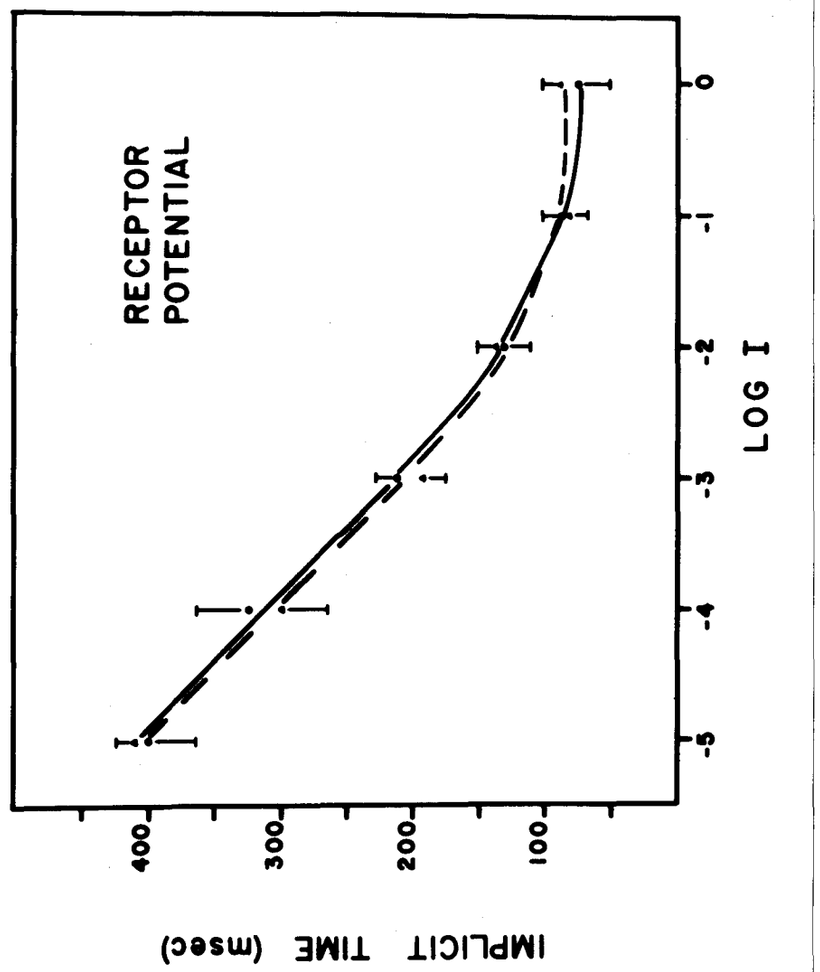


Fig. 24. Effect of picrotoxin on the implicit time of the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 7. Solid circles •—• represent mean AIMRR implicit times obtained with light stimuli in the dark in preparations superfused with control medium. Solid triangles ▲--▲ represent mean AIMRR implicit times obtained with light stimuli in the dark in preparations superfused with medium containing picrotoxin (10^{-4}M). n = 4. Vertical brackets indicate S.E.M.

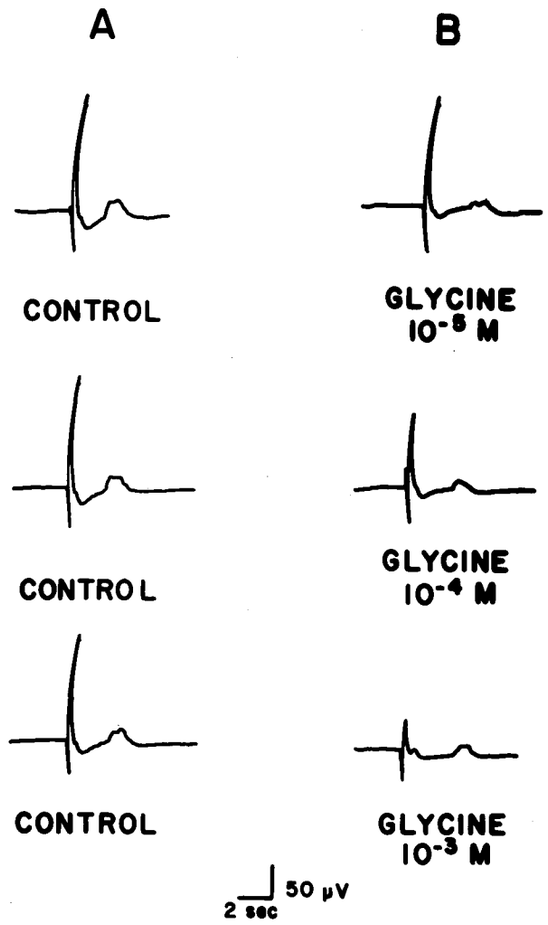


Fig. 25. Effect of glycine on b-wave amplitude. (A) control responses. (B) responses after a series of doses of glycine. The maximal effect of glycine was seen approximately 3 minutes after changing to the perfusion medium containing the drug. Recovery of responses took about 1.5 minutes after cessation of perfusion with medium containing glycine. Note the decrease in b-wave amplitude is directly proportional to glycine concentration.

At 10^{-3} M the maximum depression in b-wave amplitude produced by glycine was seen in 1 minute but recovery of control responses took about 2.5 minutes in this preparation. Fig. 26 shows the effect of glycine (5×10^{-4} M) on the R - log I curves for the b-wave both in the dark and in the presence of background light (0.008 fc). Glycine reduces the maximum response obtainable by about 45% and shifts the R - log I curve from a minimum of 0.75 to a maximum of 3.5 log units to the right. Presentation of background light further depresses the ceiling response and also shifts the R - log I curve to the right approximately 1 log unit. Overall retinal sensitivity is reduced by glycine, however, the further decrease in retinal sensitivity produced by weak background light is not altered by the drug. These results are summarized in Table 10 (Appendix B). Glycine (5×10^{-4} M), while reducing b-wave amplitude, has no significant effect on the b-wave implicit time (Fig. 27). Glycine (5×10^{-4} M) has no effect on the R - log I curve for the aspartate - isolated mass receptor response (Fig. 28) suggesting that the observed b-wave effects must be mediated by processes proximal to the photoreceptors. Fig. 29 compares the effect of strychnine and picrotoxin on glycine depression of the ERG. Neither of these drugs appears to significantly reverse this effect. The a-wave depression seen in these tracings

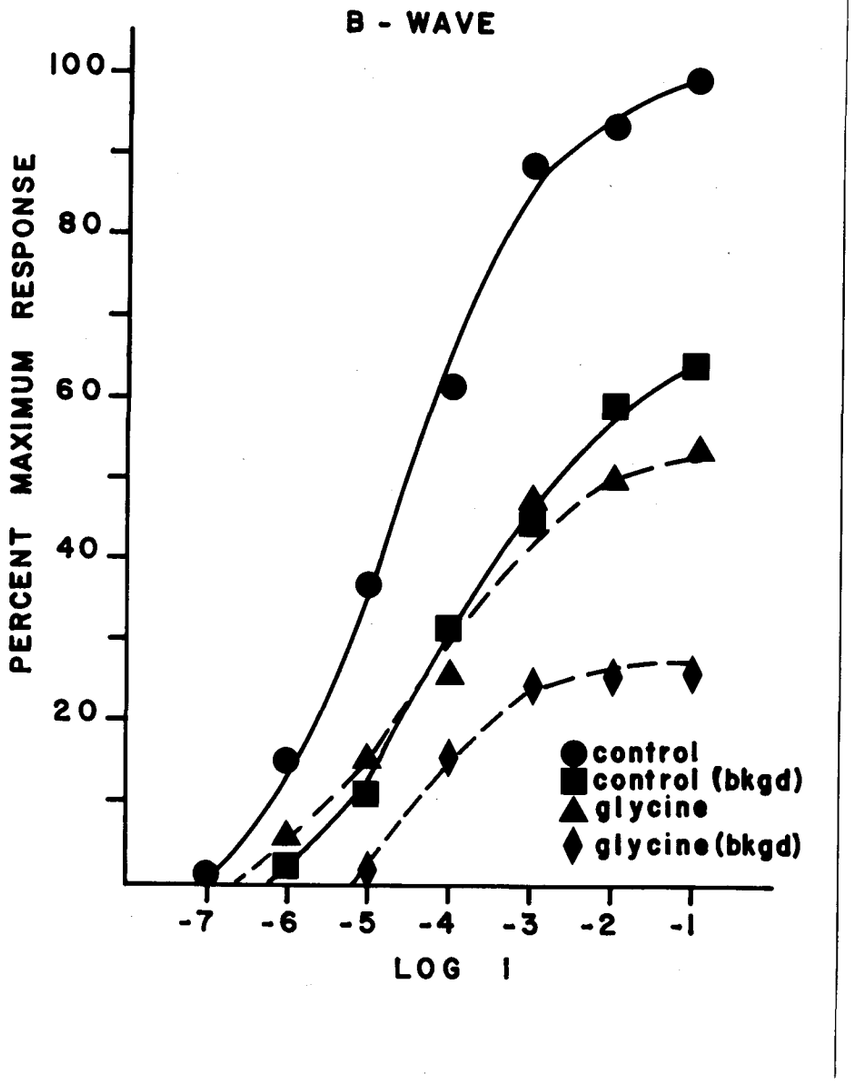


Fig. 26. Effect of glycine on the R - log I functions of the b-wave. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for control medium. Solid squares ■ represent responses to light stimuli superimposed on background light (0.008 fc). Solid triangles ▲ represent responses to light stimuli in the dark for medium containing glycine (5×10^{-4}M). Solid diamonds ◆ represent responses to light stimuli superimposed on background light (0.008 fc) for medium containing glycine (5×10^{-4}M). n = 5. Range of S.E. for data points is ± 1.0 to 7.9%.

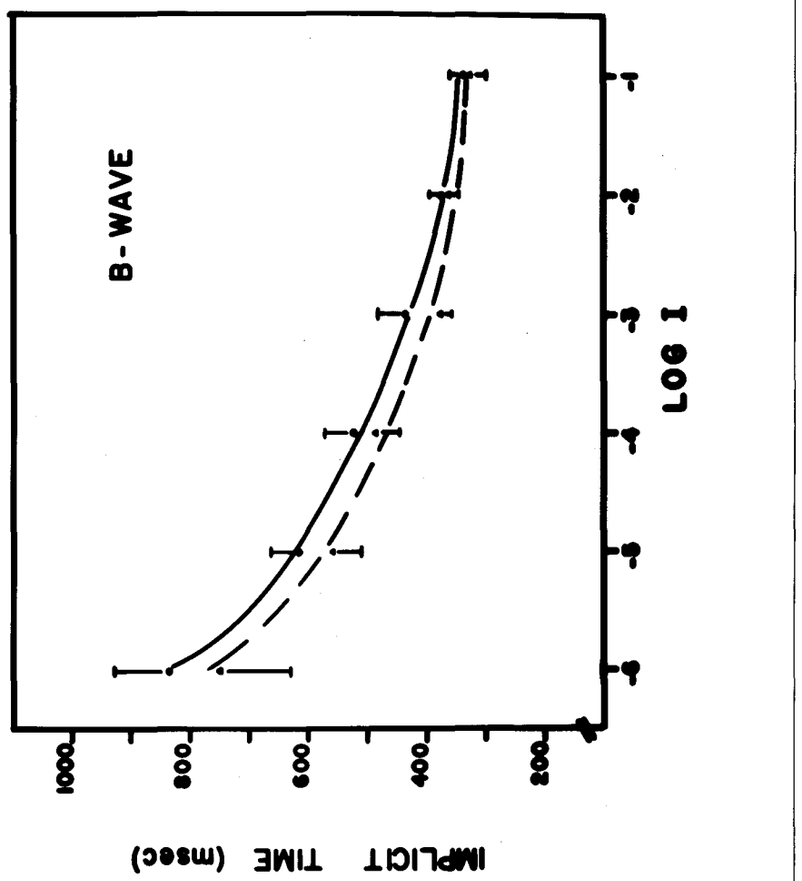


Fig. 27. Effect of glycine on the implicit time of the b-wave. The ordinate and abscissa are as described in Fig. 7. Solid circles •—• represent b-wave implicit times obtained with light stimuli in the dark for preparations superfused with control medium. Solid triangles ▲---▲ represent b-wave implicit times obtained with light stimuli in the dark with retinas superfused with medium containing glycine (5×10^{-4} M). n = 5. Vertical brackets indicate S.E.M.

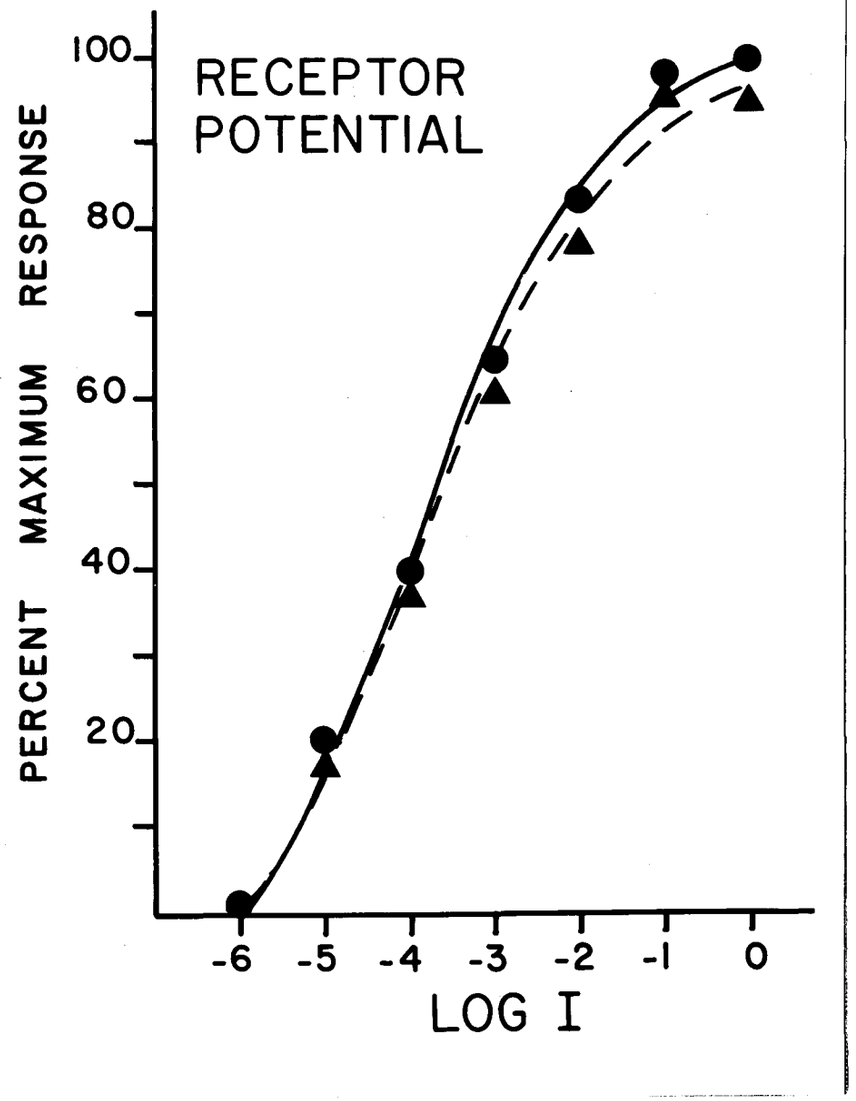


Fig. 28. Effect of glycine on the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for control medium. Solid triangles ▲ represent responses to light stimuli in the dark for preparations superfused with medium containing glycine (5×10^{-4} M). n = 3. Range of S.E. for data points is ± 0.2 to 4.6%. Responses in the presence of glycine are not significantly different from control responses.

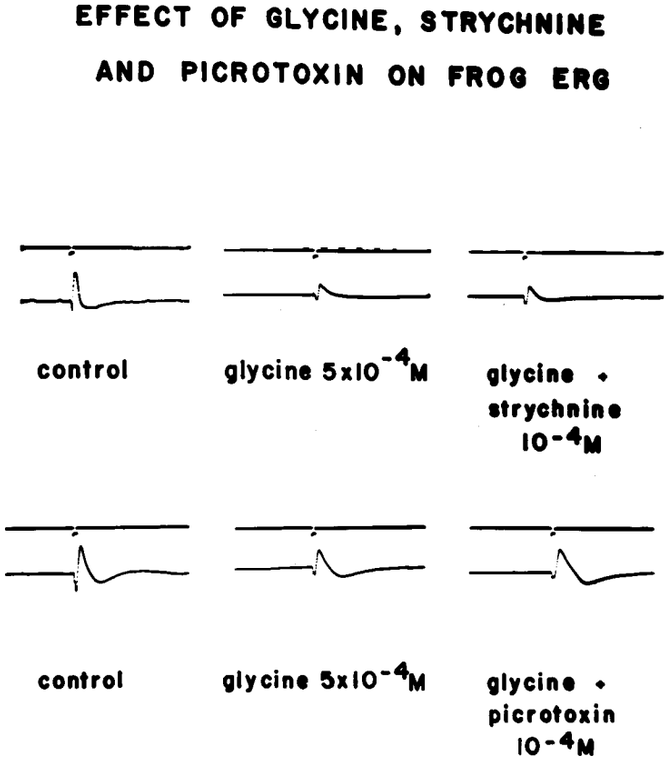


Fig. 29. Effect of glycine, strychnine and picrotoxin on the frog ERG. The upper panel shows responses to light stimuli (200 msec; -2.0 log I) with control superfusion medium, with medium containing glycine (5×10^{-4}M), and with medium containing glycine (5×10^{-4}M) and strychnine (10^{-4}M). The lower panel shows responses to light stimuli (200 msec; -2.0 log I) with control superfusion medium, with medium containing glycine (5×10^{-4}M) and picrotoxin (10^{-4}M). Note strychnine does not reverse the depression of b-wave amplitude produced by glycine. The partial reversal produced by picrotoxin may be related to its ability to increase retinal sensitivity by itself.

with glycine is not consistent and is not supported by the effect seen on the aspartate - isolated mass receptor response (cf Fig. 28).

Effect of strychnine on the ERG

Fig. 30 illustrates a representative experiment indicating that strychnine produces a depression of the electroretinogram that is proportional to dose (10^{-6} to 10^{-3} M). Fig. 31 illustrates the influence of strychnine (10^{-4} M) on the R - log I curves of the b-wave. Strychnine causes a depression of the b-wave at all stimulus intensities studied (0.0 to -4.6 log I). Presentation of background light causes a further depression of the response, but the difference between dark and background R - log I curves in the presence and absence of drug appears to be approximately the same. The results of this experiment are summarized in Table 11 - Appendix B. Strychnine (10^{-4} M) significantly increases implicit time at stimulus intensities ranging from 0.0 to -4.2 log I (Fig. 32; Table 12 - Appendix B). These increases range from 134.5% at 0.0 log I to 165.9% at -4.6 log I.

The influence of strychnine on the aspartate - isolated mass receptor response was studied to ascertain the possibility of a direct action on photoreceptors. Strychnine (10^{-4} M) neither alters the amplitude of the photoreceptor response (Fig. 33) nor the implicit time (Fig. 34) over the

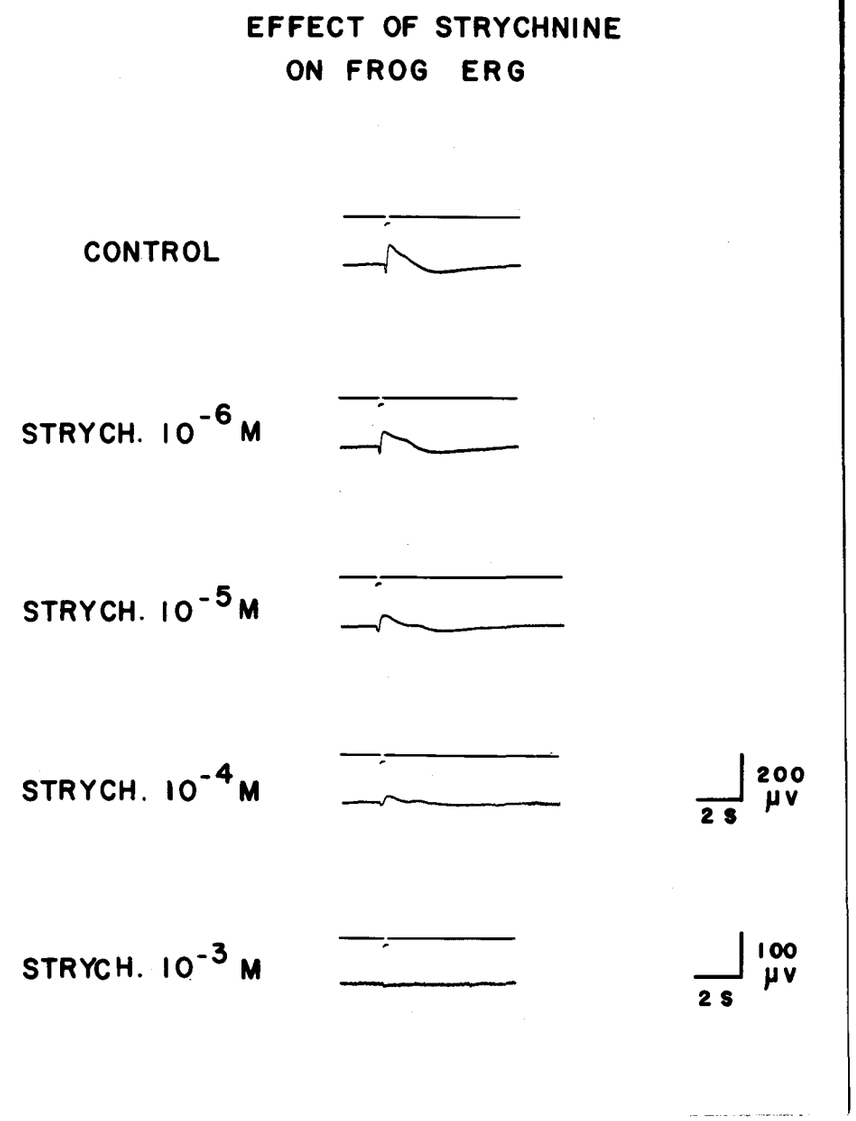


Fig. 30. Effect of strychnine on the frog ERG. The upper tracing shows a response to light stimulus (200 msec; -2.0 log I) in a preparation superfused with control medium. The lower tracings show responses to light stimuli (200 msec; -2.0 log I) with medium containing various doses of strychnine. Note the decrease in response is directly proportional to the dose of strychnine over a range of 10^{-6} to 10^{-3}M.

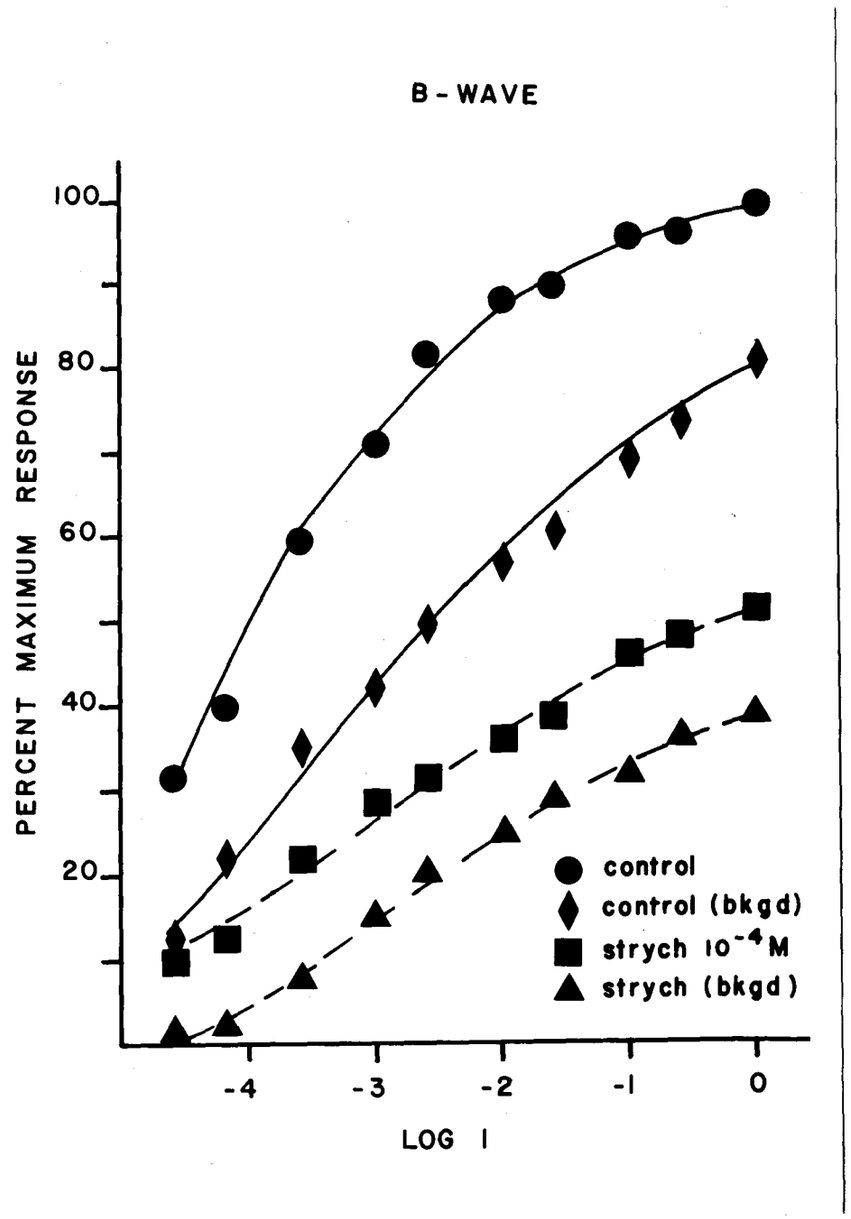


Fig. 31. Effect of strychnine on the R - log I functions of the b-wave. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light in the dark for control medium. Solid diamonds ◆ represent responses to light stimuli superimposed on background light (0.008 fc). Solid squares ■ represent responses to light stimuli in the dark for medium containing strychnine (10^{-4}M). Solid triangles ▲ represent responses to light stimuli superimposed on background light (0.008 fc) for medium containing strychnine (10^{-4}M). n = 6. Range of S.E. for data points is ± 0.8 to 6.5%.

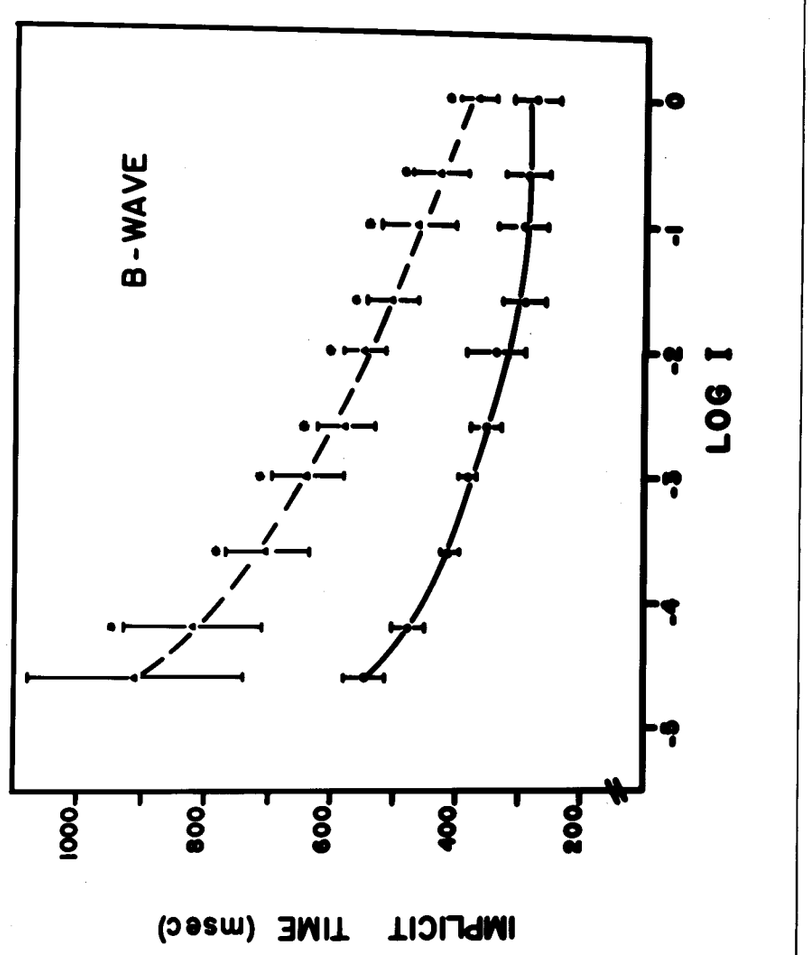
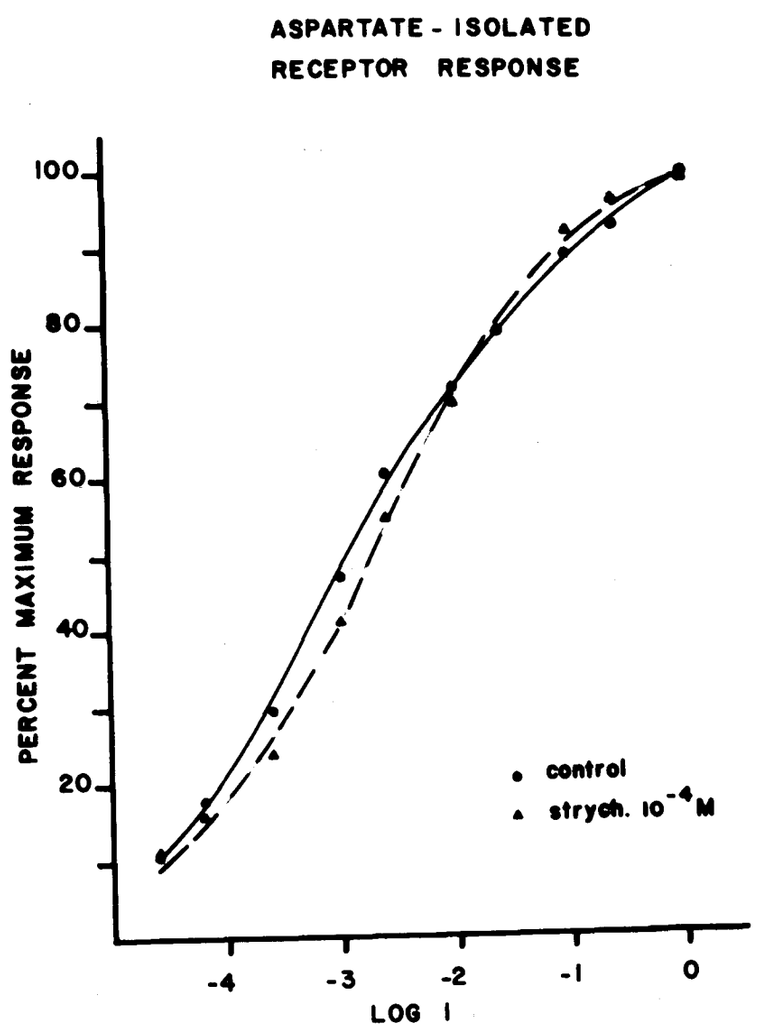


Fig. 32. Effect of strychnine on the implicit time of the b-wave. The ordinate and abscissa are as described in Fig. 7. Solid circles •—• represent b-wave implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲---▲ represent b-wave implicit times obtained with light stimuli in the dark with retinas superfused with medium containing strychnine (10^{-4}M). n = 5. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$).

Fig. 33. Effect of strychnine on the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 5. Solid circles •—• represent responses to light stimuli in the dark for preparations superfused with control medium. Solid triangles ▲---▲ represent responses to light stimuli in the dark for retinas superfused with medium containing strychnine (10^{-4} M). n = 4. Range of S.E. for data points is ± 4.4 to 17.8%. Responses in the presence of strychnine are not significantly different from control responses.



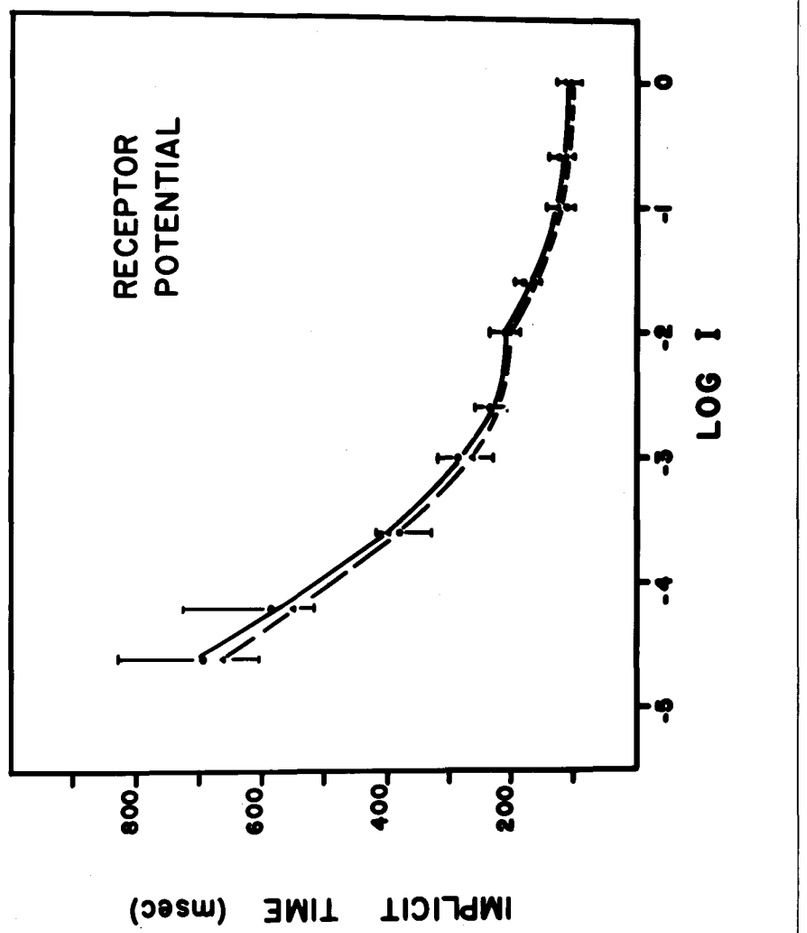


Fig. 34. Effect of strychnine on the implicit time of the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 7. Solid circles •—• represent AIMRR implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲---▲ represent AIMRR implicit times obtained with light stimuli in the dark for medium containing strychnine (10^{-4} M). n = 4. Vertical brackets indicate S.E.M. Responses in the presence of strychnine are not significantly different from control responses.

entire range of stimulus intensities studied.

Dopamine and light adaptation

Dopamine has been shown to have inhibitory activity in the retina in other species. It was of interest, therefore, to investigate the possibility of its involvement in the reduction of sensitivity produced by background light. No effect on the frog retina electroretinogram was seen at dopamine doses of 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M. Furthermore, no effect on the R - log I curves of the b-wave in dark- or light-adapted conditions was seen after dopamine (Fig. 35; Table 13 - Appendix B). Similarly, the b-wave implicit time is unaltered by this drug (Fig. 36). An examination of the aspartate - isolated mass receptor response in the presence and absence of this agent indicated it was without effect. Fig. 37 shows the mean R - log I curve generated in the dark for two animals. Neither an alteration in amplitude nor a change in implicit time was observed over the range of stimulus intensities used.

Delayed off-response

Photic stimulation of the isolated frog retina elicits an electroretinogram (ERG) with its characteristic a- and b-waves. A delayed off-response, or e-wave, appearing seconds after the a- and b-waves have disappeared can also be elicited under appropriate conditions; that is, stimulus

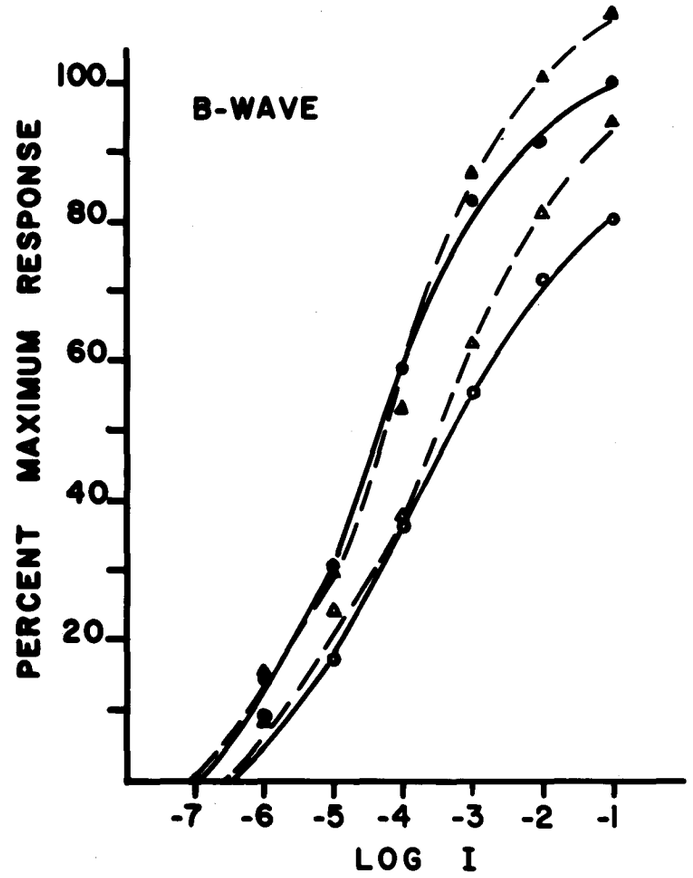


Fig. 35. Effect of dopamine on the R - log I functions of the b-wave. The ordinate and abscissa are as described in Fig. 5. Solid circles ● represent responses to light stimuli in the dark for preparations superfused with control medium. Open circles ○ represent responses to light stimuli superimposed on background light (0.008 fc) for control medium. Solid triangles ▲ represent responses to light stimuli in the dark for retinas superfused with medium containing dopamine (10^{-4} M). Open triangles △ represent responses to light stimuli superimposed on background light (0.008 fc) for medium containing dopamine (10^{-4} M). n = 4. Range of S.E. for data points is ± 0.9 to 15.7%.

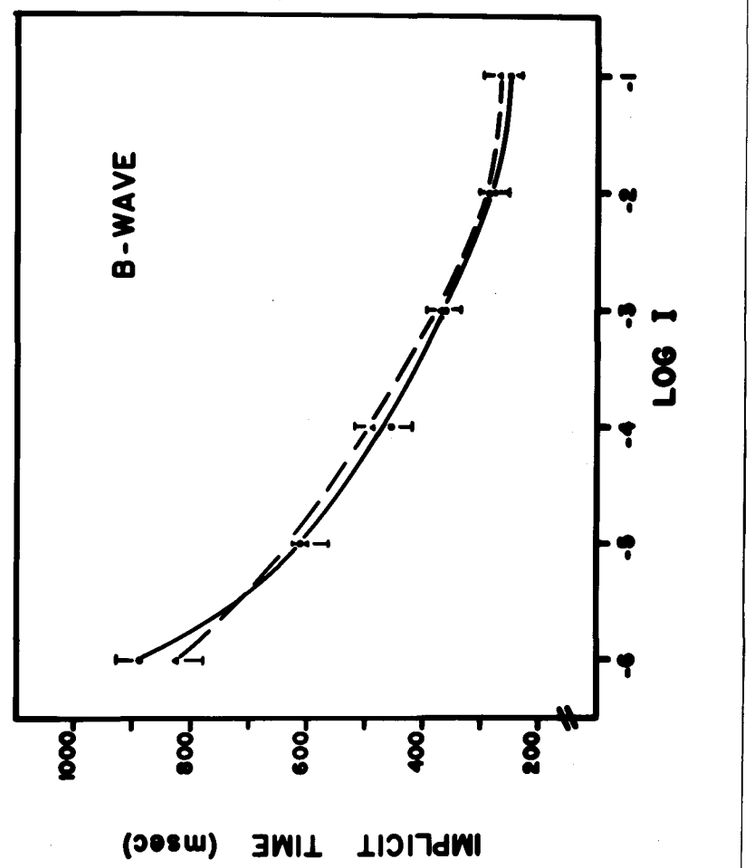


Fig. 36. Effect of dopamine on the implicit time of the b-wave. The ordinate and abscissa are as described in Fig. 7. Solid circles ●—● represent b-wave implicit times obtained with light stimuli in the dark for control medium. Solid triangles ▲---▲ represent b-wave implicit times obtained with light stimuli in the dark for retinas superfused with medium containing dopamine (10^{-4} M). n = 4. Vertical brackets indicate S.E.M. These curves do not differ significantly from each other.

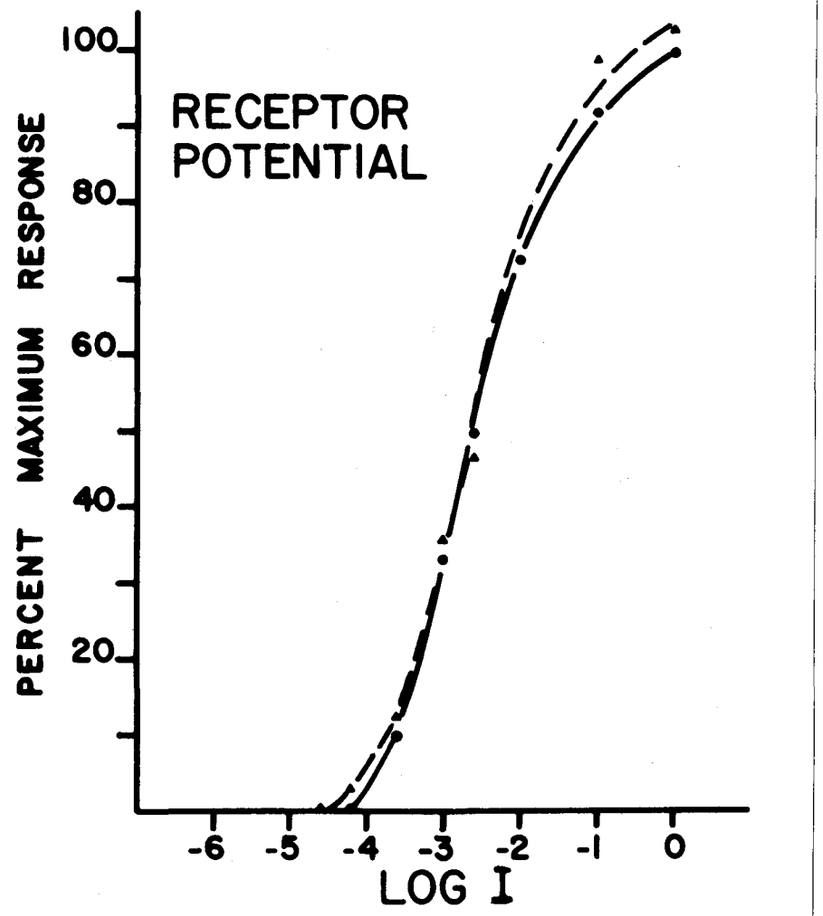


Fig. 37. Effect of dopamine on the aspartate - isolated mass receptor response. The ordinate and abscissa are as described in Fig. 5. Solid circles •—• represent responses to light stimuli in the dark for preparations superfused with control medium. Solid triangles ▲---▲ represent responses to light stimuli in the dark for retinas superfused with medium containing dopamine (10^{-4} M). n = 2. These curves do not differ significantly from each other.

durations up to 2 seconds and intensities less than -1.0 log I (where intensity of illumination is no greater than 1.33 mW/cm^2). Examination of the e-wave was included in this study of light-adaptation because of the possible involvement of this response with light- and dark-adaptation (Crescitelli and Sickel, 1968). In retinas isolated from dark-adapted animals the delayed off-response was observed at stimulus intensities capable of eliciting b-waves at the upper portion of the R - log I curve; that is, at stimulus intensities greater than -3.0 log I. The amplitude of the e-wave is inversely related, while the time - to - peak (TTP) of this response is directly related to stimulus intensity. This relationship is demonstrated in tracings from a representative experiment shown in Fig. 38. With a stimulus intensity of 9 $\mu W/cm^2$, the e-wave amplitude is 90 μV. With an 11 fold increase in intensity, the e-wave amplitude is reduced to 55 μV while the TTP increases 52%; that is, from 13.8 to 21.0 seconds. The duration of light stimulation also influences the e-wave. Increasing stimulus duration over a range of 10 msec to 2 sec significantly increases the TTP of this response and decreases its amplitude. Fig. 39 shows tracings from a typical experiment in which this phenomenon is demonstrated. With stimulus at a constant intensity (99 $\mu W/cm^2$), the e-wave amplitude is maximal with a

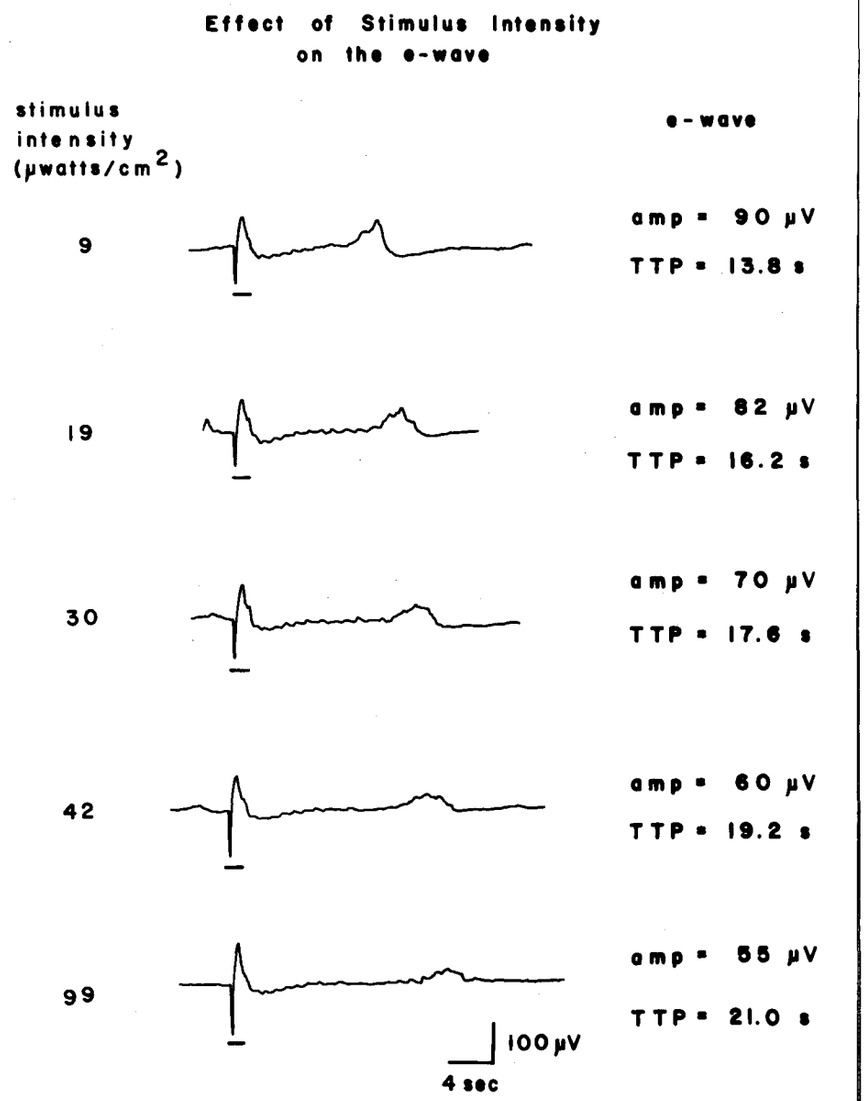


Fig. 38. Effect of stimulus intensity on the e-wave of the ERG. Responses to white light stimulation with a constant duration of 1 second are shown for increasing stimulus intensities. The line below the tracing indicates the stimulus duration. Note the decrease in e-wave amplitude but the increase in its time - to - peak (TTP) with increasing stimulus intensity.

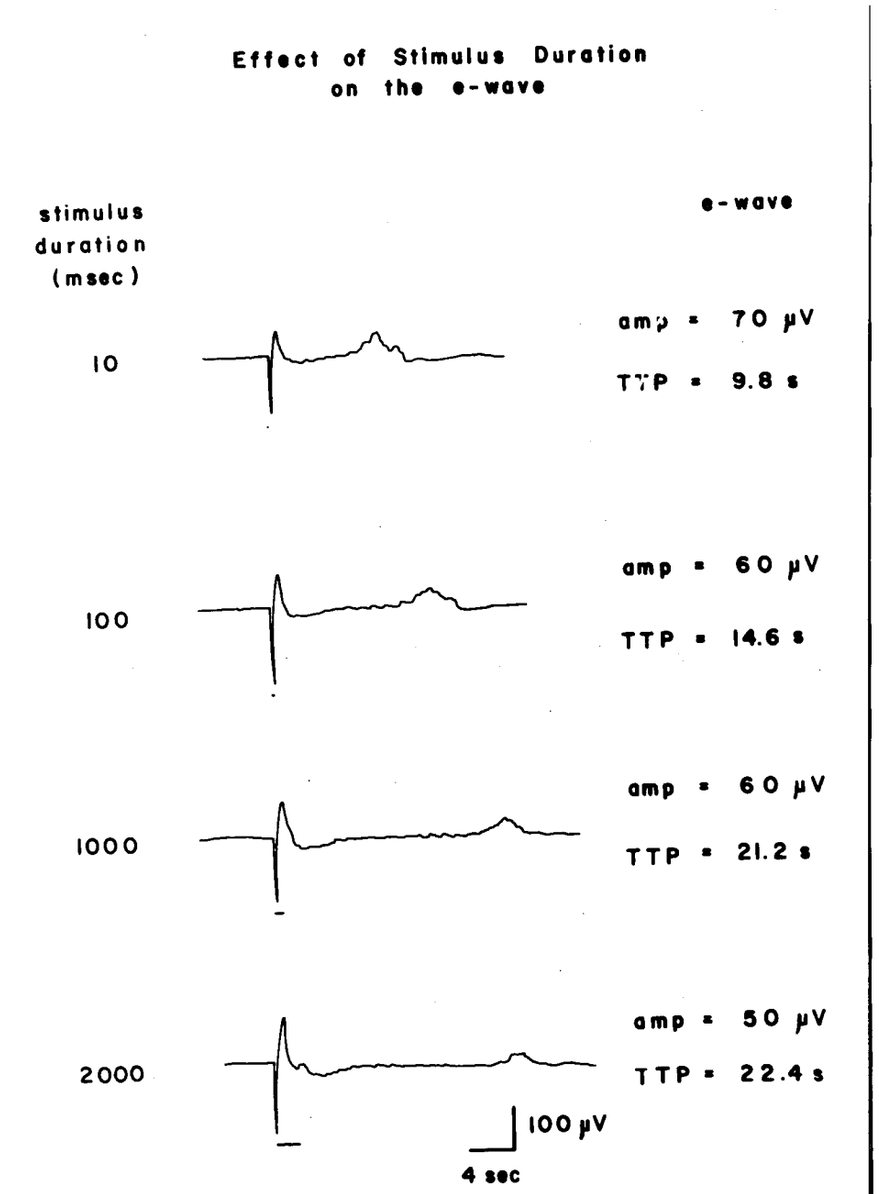


Fig. 39. Effect of stimulus duration on the e-wave of the ERG. Responses to white light stimulation with a constant intensity of 99 $\mu W/cm^2$ are shown for increasing stimulus durations. The line below the tracing indicates the stimulus duration. Note the decrease in e-wave amplitude but the increase in its time - to - peak (TTP) with increasing stimulus duration.

10 msec stimulus and falls to about 29% of this level when the duration is increased to 2 sec. The TTP, on the other hand, at a stimulus duration of 2 sec. is 228% of that found at 10 msec.

The e-wave is responsive to changes in the spectrum of the stimulus. This is observed by shifting the wavelength of light stimulation toward the blue or red end of the visible spectrum, which permits one to determine changes resulting from rod or cone activity (cf Table 14 - Appendix B). The amplitude of the e-wave is unchanged by shifts in stimulus wavelength. However, the time - to - peak of the response shortens significantly when the stimulus is shifted to the red end of the visible spectrum. Neither a-wave nor b-wave amplitudes differ significantly with red, white or blue stimuli.

Several putative inhibitory transmitters endogenous to the retina were studied for their effects on the delayed off-response. The results of an experiment in which GABA (10^{-4}M) was administered are shown in Fig. 40. B-wave amplitude was significantly reduced ($p<0.05$ in each instance) by GABA. The amplitude of the delayed off-response significantly increased: 40.6% at 400 nm, 54.0% at 650 nm and 20.2% with white light. The slight increase in time - to - peak of these responses was not statistically significant (cf

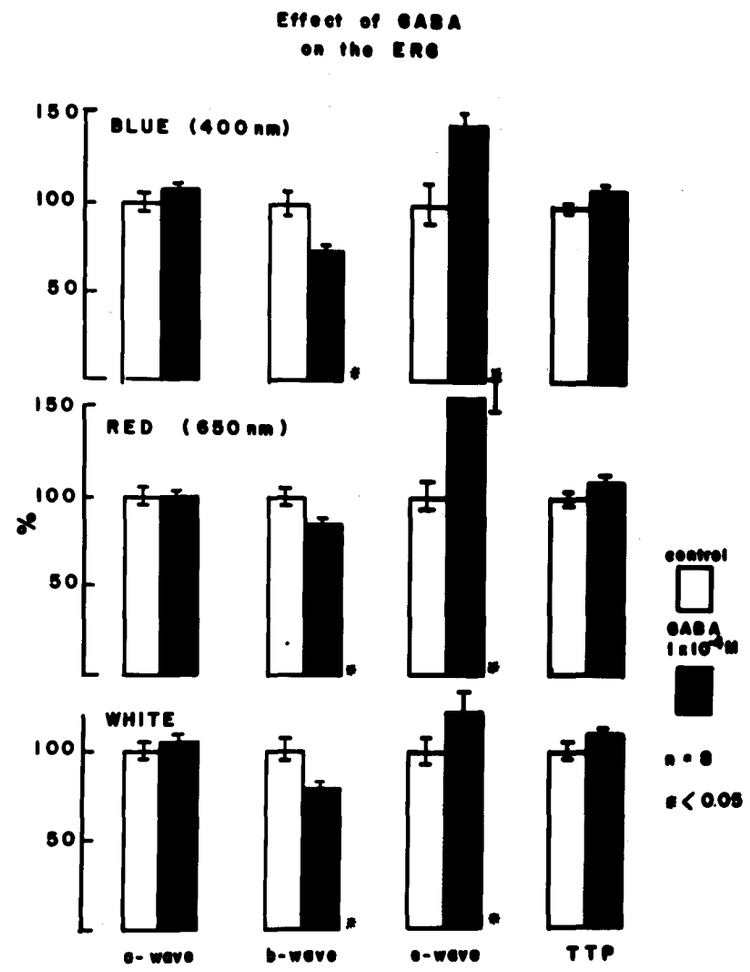


Fig. 40. Effect of GABA on the ERG. The ordinate indicates response as per cent of control values. The abscissa indicates a-, b- and e-wave amplitude and time - to - peak (TTP) of the e-wave. Stimulus duration was 200 msec. The stimulus was either white, blue (400 nm) or red (650 nm) light. Clear bars represent control responses. Shaded bars represent responses obtained after GABA (10^{-4}M). n = 8. Vertical bars indicate S.E.M. * indicates significant difference from control. Note GABA significantly increases e-wave amplitude without changing its TTP. B-wave amplitude is reduced while that of the a-wave is unaltered by GABA administration.

Table 15 - Appendix B). GABA did not modify a-wave amplitude. GABA effects on the various components of the ERG were not significantly altered by changes in stimulus wavelength.

Bicuculline (2×10^{-4}M), a GABA antagonist, significantly reduced e-wave amplitude from control responses at all stimulus wavelengths studied (Fig. 41; Table 16 - Appendix B). With white light stimulation the bicuculline induced depression of e-wave amplitude was approximately 25%. The TTP was unaltered by the drug and differences in spectral sensitivity were undetectable. Bicuculline, at the concentration used, was without effect on a-wave amplitude regardless of stimulus wavelength. Bicuculline significantly reduced b-wave amplitude only when stimulus wavelength peaked in the blue.

Glycine (5×10^{-4}M) depresses the neural retina (Fig. 42). The amplitudes of the b-wave and the e-wave are significantly reduced (22 and 27% respectively). The a-wave and the TTP of the e-wave, however, are not significantly different from controls.

Dopamine, contrary to expectations, had no detectable effect on the ERG. The results of experiments in which the retina was superfused with dopamine at 10^{-6} and 10^{-5} M are shown in Fig. 43. No alterations in a-, b- and e-wave am-

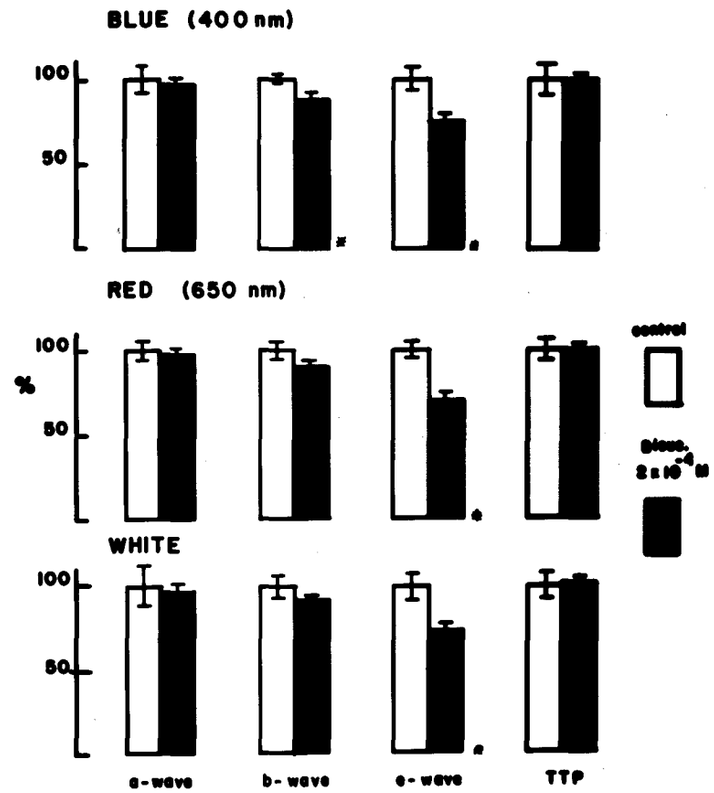


Fig. 41. Effect of bicuculline on the ERG. The ordinate indicates response as per cent of control values. The abscissa indicates a-, b- and e-wave amplitude and time - to - peak (TTP) of the e-wave. Stimulus duration is 200 msec. The stimulating light is white, blue (400 nm) or red (650 nm). Clear bars indicate control responses. Shaded bars show responses obtained after bicuculline (2×10^{-4} M). n = 8. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$). Note bicuculline significantly decreases e-wave amplitude without changing its TTP.

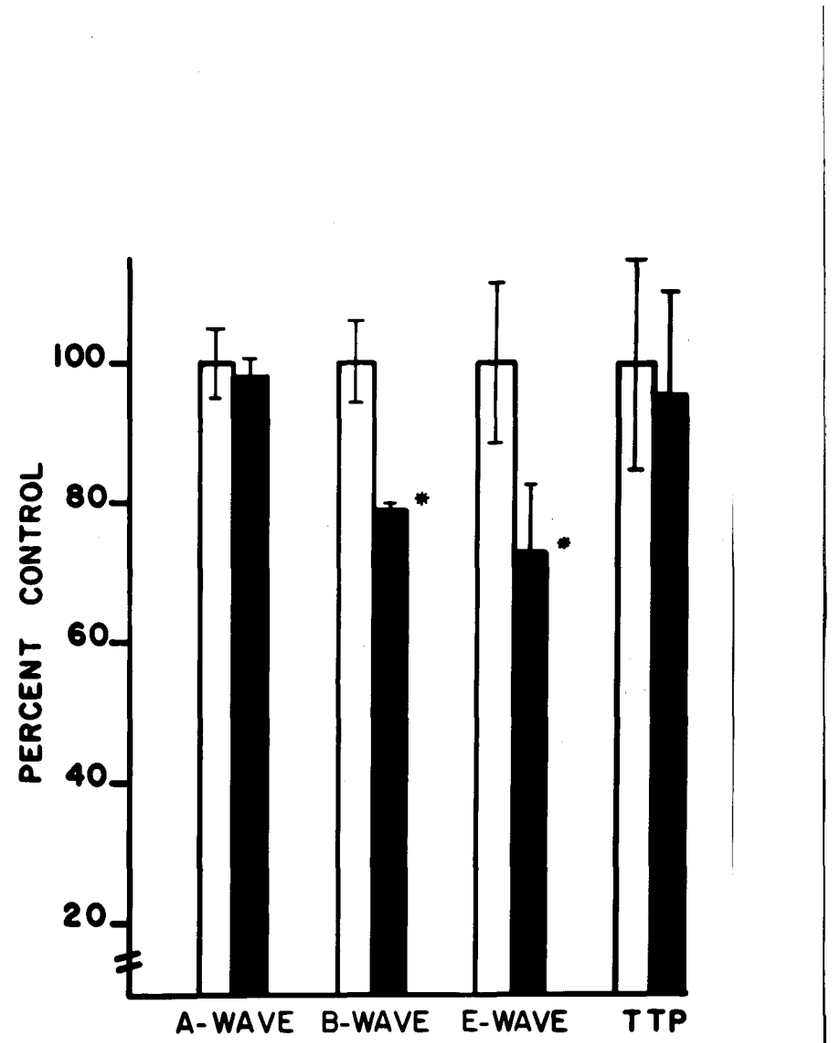


Fig. 42. Effect of glycine on the ERG. The ordinate and abscissa are as described in Fig. 40. White light stimulus with a pulse duration of 200 msec was used. Clear bars indicate control responses. Shaded bars show responses obtained after glycine (5×10^{-4}). n = 3. Vertical brackets indicate S.E.M. * indicates significant difference from control ($p < 0.05$). Note glycine administration reduced both b- and e-wave amplitude but did not alter e-wave time - to - peak (TTP).

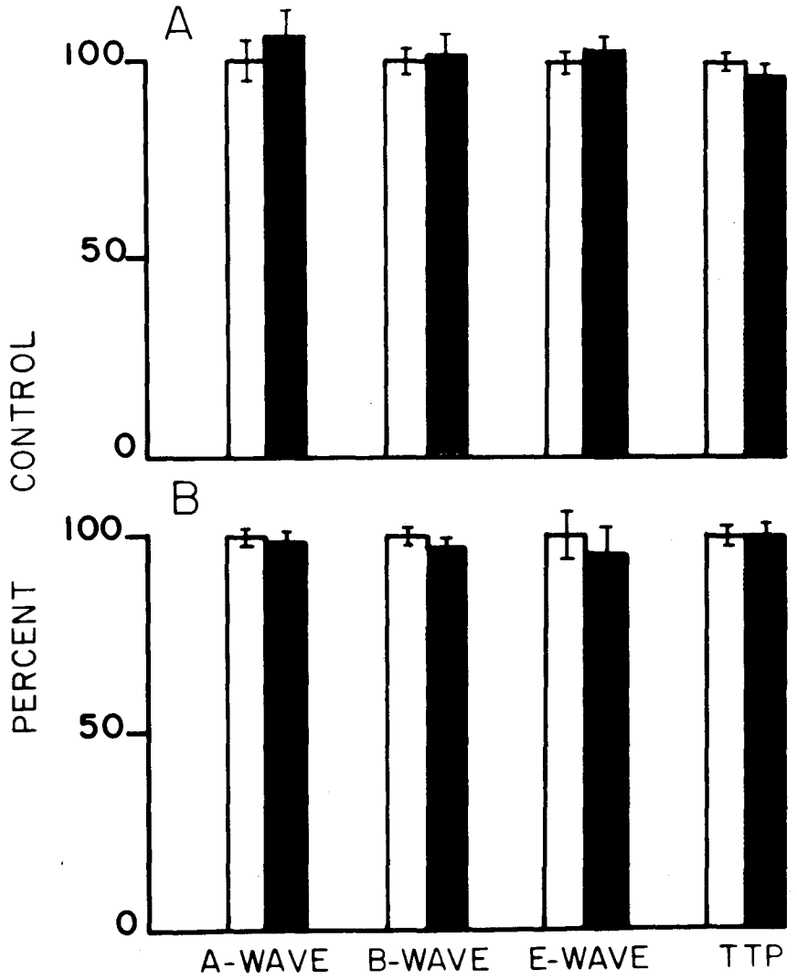


Fig. 43. Effect of dopamine on the ERG. The ordinate and abscissa are as described in Fig. 40. White light stimulus with a pulse duration of 200 msec. was used. Clear bars indicate control responses. Shaded bars show responses obtained after dopamine at 10^{-5} M (A) and 10^{-6} M (B). n = 5. Vertical brackets indicate S.E.M. Note dopamine has no effect on a-, b- or e-wave amplitudes nor on e-wave time - to - peak (TTP).

plitude nor in the TTP of the e-wave was obtained, nor did these occur at concentrations as high as 10^{-3} M (not illustrated).

The effect of GABA and picrotoxin was examined in bleached retinas to determine the extent to which light treatment modified these responses. After control responses were recorded, the retinas were bleached with full field stimulus light at -2.0 log I for 30 minutes. Subsequently, the retinas were returned to darkness and allowed to recover for approximately 20 minutes. Responses were observed in the presence and absence of GABA or picrotoxin. In tracings from a representative experiment (Fig. 44) it is shown that GABA depresses b-wave amplitude but is without significant effect on e-wave amplitude or TTP. These effects are reversible by washing out the GABA with control medium. The GABA antagonist picrotoxin, on the other hand, reduced e-wave amplitude. Like GABA itself, it is without significant effect on the TTP. This response is similar to the effect seen previously with another GABA antagonist, bicuculline, in the unbleached retina (cf Fig. 41).

In microelectrode studies in two animals an apparent correlation between the delayed off-response and ganglion cell spiking activity was observed. Fig. 45 depicts a histogram of ganglion cell spiking activity and superimposed

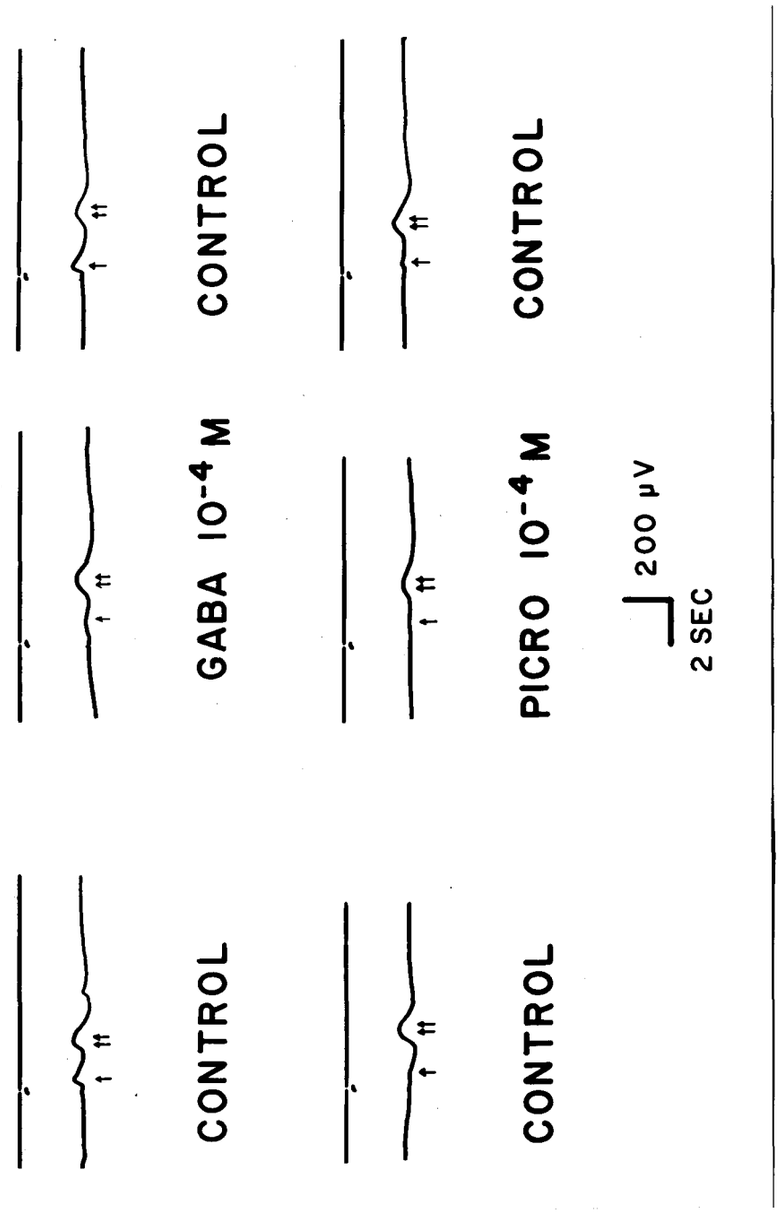


Fig. 44. Effect of GABA and picrotoxin on the ERG elicited from bleached retinas. Upper panel shows control response to light stimulation (200 msec; -4.0 log I), response in the presence of GABA (10^{-4} M) and after GABA is washed out and the preparation has recovered. Lower panel shows control response to light stimulation, after picrotoxin (10^{-4} M), and following recovery. Light stimulus is indicated by the line above each tracing. Note GABA depresses b-wave (↑) amplitude and slightly increases e-wave (↑↑) amplitude. Picrotoxin decreases e-wave (↑↑) amplitude.

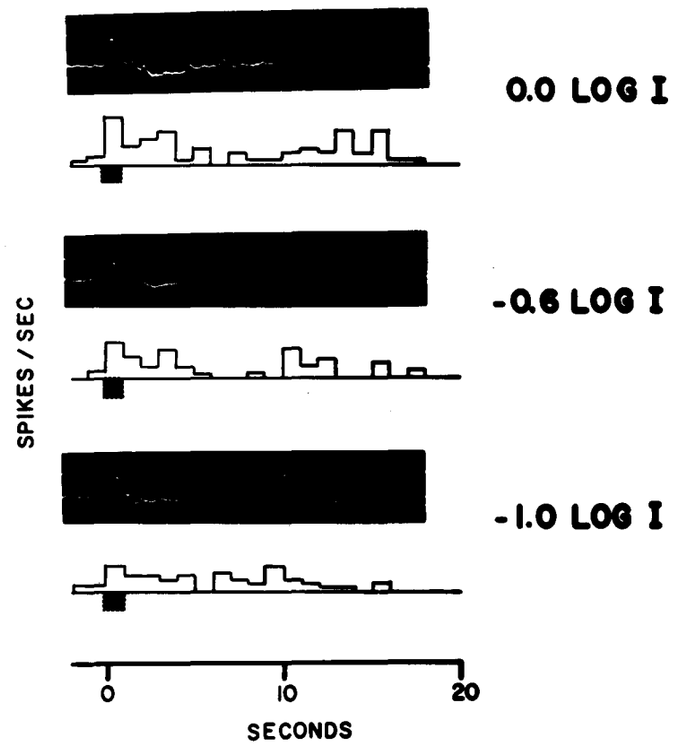


Fig. 45. Correlation between ganglion cell spiking activity and slow potential changes. The histogram represents spikes per 1 second intervals. Superimposed on the histograms are tracings of slow potential changes recorded with microelectrodes using identical stimulus parameters as were used to generate the histogram. Stimulus duration was 1 sec, while intensity was varied from 0.0 log I to -1.0 log I.

tracings of the corresponding slow potential changes occurring in response to light stimulation of decreasing intensity. The amplitude of the e-wave is increased as stimulus intensity decreases, while the TTP of this response characteristically is decreased. Corresponding to the appearance of the e-wave is a burst of ganglion cell activity. Decreased spiking activity occurs with increased e-wave amplitude. The spike activity occurring during light stimulation appears to be directly proportional to stimulus intensity in the ganglion cells.

Ganglion cell activity

Ganglion cell activity was recorded with microelectrodes because these cells, which do not contribute to the ERG, cannot be monitored by means of gross potential changes. 25 cells from 13 different animals were isolated for periods sufficiently long to characterize them as to type and responsiveness to light stimulation. Approximately 80% of these cells were spontaneously-firing cells and did not respond to full field stimulation. The remaining cells did respond to stimulation but only at stimulus intensities ranging from -3.0 to -1.0 log I. These intensities clearly are at the upper portion of the b-wave R - log I curve and are characteristic of all those cells which did respond to full field stimulation. It was not possible to generate R - log

I curves for ganglion cell activity with the full field light stimulation used in these experiments.

Survey of drugs

Superfusion of the frog retina with ACh had no effect on the ERG, except at very high concentrations (1 mM) where a small depression of a- and b-wave amplitude was observed. Similarly, physostigmine (10^{-5} M) did not alter these light-evoked field potentials. There is evidence that ACh has a transmitter function in the retina (Ames and Pollen, 1969; van Harreveld, 1976) although the results of the present study do not lend support to this idea.

In a like manner, norepinephrine was found to be without effect on the ERG. Dopamine, which is the principal catecholamine in the vertebrate retina, also does not alter a- and b-wave amplitudes. It is conceivable that catecholaminergic pathways are present in the retina but that their influence on retinal field potentials can not be demonstrated by the stimulus parameters used in the present study.

Serotonin (10^{-5} M) produced a moderate increase in a- and b-wave amplitudes. This enhancement of neuronal activity is in keeping with the increase in spontaneous and light-evoked ganglion cell activity produced by serotonin (5×10^{-6} M) in the isolated rabbit retina (Ames and Pollen,

1969). The fact that melatonin (10^{-5} M) also produces a small increase in b-wave amplitude is interesting since the retina is the only vertebrate tissue besides the pineal which has been shown to have hydroxyindole - o - methyl transferase activity, necessary for the conversion of serotonin to melatonin (Graham, 1974).

Taurine levels are very high in the vertebrate retina and this amino acid appears to be associated primarily with photoreceptor cells (Cohen et al., 1973). Taurine (10^{-3} M) produced a depression in b-wave amplitude while having no effect on the a-wave, which is similar to that reported previously in chicken (Pasantes-Morales et al., 1973) and rabbit (Starr, 1975b). The fact that taurine at 10 - 100 μM produced an increase in b-wave amplitude is not readily explainable since 100 μM taurine has been shown earlier to cause a decrease in b-wave amplitude in the isolated frog retina (Urban et al., 1976).

Histamine (10^{-5} M) produced a moderate increase in a- and b-wave amplitudes, while histidine (10^{-5} M) also produced an increase although not as great. While Sugawara and Negishi (1973a) observed no effect of histidine on horizontal cells in the isolated carp retina, I know of no previous report on the effect of histamine on the vertebrate retina.

The depression of a- and b-wave amplitudes seen with phenobarbital at high concentration (5 mM) and enhancement of b-wave amplitude at a lower concentration (1 mM) is in keeping with the effect of Na - pentothal on isolated frog retina observed previously (Rockstroh and Hanitzsch, 1966). In general, barbiturates appear to follow this pattern in their influence on the ERG (van Harreveld, 1976).

The effects of these agents, together with several other drugs whose influence on the frog ERG are less consistent, are summarized in Appendix C.

DISCUSSION

In attempting to analyze physiological processes in the retina, it is convenient to think of light information as being processed and transmitted to more central areas of the brain by a three neuron chain: photoreceptor, bipolar and ganglion cells. Measuring responses from each of these groups of cells separately can give an indication of the type of information processing that is occurring at each level in the retina.

The photoreceptors respond to light stimulation by slow, graded potentials rather than by generating spikes. The activity of individual cells, therefore, must be measured using intracellular recording techniques. The difficulty in recording from these small cells for long periods of time and under conditions in which the chemical environment of the tissue is to be manipulated has made this approach unacceptable for certain studies. Furukawa and Hanawa (1955) demonstrated that the application of sodium aspartate to the toad retina suppressed the PII component of the ERG and allowed for the external recording of the mass PIII component. Sillman et al. (1969) described this response in greater detail using isolated frog retina. They showed that the mass receptor potential remaining after

treatment with sodium aspartate (10 to 110 mM) consists almost exclusively of the receptor response or distal PIII. Furthermore, both rods and cones contribute to this response. The aspartate-isolated mass receptor response was used in the present experiments because it is considered a good index of receptor activity (Normann and Werblin, 1974). The R - log I curves for this response show a threshold in the dark at approximately -5.0 log I, with a "working range" that extends about 3 log units under scotopic conditions. The range of responsiveness of photoreceptors measured in our experiments is in keeping with that measured previously in Necturus (Normann and Werblin, 1974) and skate (Green et al., 1975). Furthermore, the biphasic waveform of the AIMRR, which can be altered with changes in stimulus intensity, is suggestive of both rod and cone contribution to this response, an idea which was presented earlier from experiments involving spectral sensitivity curves (Sillman et al., 1969).

Cells comprising the inner nuclear layer of the retina also respond to light stimulation with slow, graded potentials. The synchronous response to light by the population of cells making up this layer can be quantified by measurement of the amplitude of the b-wave of the ERG. Comparing intracellular responses from all cells in the inner nuclear layer with the b-wave suggests that the site of ori-

gin of the b-wave is actually the glial cells (Muller cells) which extend through the inner nuclear layer (Miller and Dowling, 1970). It is postulated that the Muller cells depolarize in response to increases in extracellular K^+, a result of neuronal activity. This wave of depolarization is seen as the b-wave, which acts as a good index of inner nuclear layer activity (Dowling, 1970; Armington, 1974). The R - log I curves for the b-wave in our experiments show a threshold in the dark at approximately -6.0 log I, one log unit lower than that of the aspartate - isolated mass receptor response. This is in keeping with previous investigations (Green et al., 1975) and undoubtedly reflects summation occurring at the second order neurons. Like the AIMRR, the "working range" of the b-wave extends approximately 3 log units under scotopic conditions. Presentation of background light increases the threshold for this response and shifts the R - log I curves to the right, indicating decreased retinal sensitivity. Since neuronal activity in the inner nuclear layer is dependent on synaptic interaction, it was felt that the b-wave would be a good measure of retinal response to various putative neurotransmitters and their antagonists.

The ganglion cell layer consists of cells which are essentially third-order neurons in the chain of information

transfer to the brain. When stimulated by light these cells respond with action potentials superimposed upon a slight depolarization, as measured with intracellular electrodes (Werblin and Dowling, 1969). Since their contribution to field potentials has been shown to be small, they contribute little to the ERG (Sickel, 1972). Measurement of changes in neuronal activity of the ganglion cells during light-adaptation was attempted by monitoring spike activity of individual cells in response to diffuse full field light stimulation. Although this approach has been successfully applied to the skate (Green et al., 1975) and Necturus (Werblin and Dowling, 1969), it was observed in these studies that this type of stimulation was incapable of eliciting responses in most ganglion cells of the isolated frog retina. Previous studies have used diffuse illumination when measuring activity of frog ganglion cells (Naka et al., 1960), but this type of stimulation is apparently not suitable for demonstrating changes in R - log I curves associated with weak background illumination.

Ganglion cells in the frog have been classified by Maturana et al. (1960) into the following functional types: (1) sustained edge detectors, (2) convex edge detectors, (3) changing contrast detectors, (4) dimming detectors, and (5) dark detectors. It is apparent that most of the cells

observed in the present experiments were primarily spontaneously firing cells insensitive to diffuse illumination. These may correspond to the dark detectors of the aforementioned classification. Responses could be elicited from the remaining cells only with very intense light flashes. It is felt that only stimulation which takes advantage of the unique properties of the different functional classes of ganglion cells would lead to responses suitable for measurement of changes associated with adaptation to weak background illumination. The highly specific response of frog ganglion cells is characteristic of the frog "complex" retina. Other species, for example the monkey and the cat, show a "simple" type of ganglion cell receptive field organization and are, therefore, more responsive to non-specific stimulation.

The time-course as well as the amplitude of field potential responses is an important parameter for assessing neuronal activity. In the case of the ERG and the aspartate-isolated mass receptor response, the time-course, or implicit time, of each component is taken as the time from the beginning of stimulation to the peak of the response. The implicit times for these responses are very long, ranging from 100 msec to approximately 1 second, depending on the conditions of stimulation. These times reflect the slow

potential changes associated with light stimulation which is characteristic of the distal portions of the retina. For most field potential responses the implicit time decreases with increasing stimulus intensity. In these experiments the implicit time of the ERG b-wave showed a linear decrease with a log stimulus intensity increase. In the presence of weak background light the b-wave implicit time is increased at stimulus intensities in which the rods might be expected to dominate the response. This could be interpreted as an inhibition of cone controlled neuronal activity and a shift toward a characteristically slower rod response. At high stimulus intensities this would not be seen because of the predominance of cone activity. This type of transition from rod to cone responses is more evident when the implicit times of the aspartate-isolated mass receptor response are examined. The decrease in implicit time with increasing stimulus intensity describes a curve containing two distinct components (Fig. 7 and 8; see also Appendix D for mathematical proof). At lower stimulus intensities (< -2.0 log I) the implicit time decreases rapidly in exponential fashion as log I increases until it reaches a plateau. A second component reflecting a slower rate of decrease in implicit time occurs at higher stimulus intensities (> -2.0 log I); that is, the point at which cones are beginning to

dominate the response. This transition from rod to cone activity is also manifested by a change in waveform of the aspartate-isolated mass receptor response (Fig. 10A) and the a-wave of the ERG (Fig. 10B) at a stimulus intensity of -2.0 log I. The biphasic waveform of the AIMRR is attributed to fast cone and slow rod contributions to this potential. This has been previously reported in cat and monkey (Brown, 1968), Necturus (Normann and Werblin, 1974) and, most recently, in frog (Hemila, 1977). Furthermore, simultaneous intracellular recording of cone and rod responses in turtle has given support to this idea (Copenhagen and Owen, 1976).

Light- and dark-adaptation

Visual adaptation is that process by which the eye adjusts itself to changes in ambient light levels ranging over a span as great as 10 log units. Dark-adaptation has been studied extensively under conditions in which the eye is removed from relatively strong background illumination and placed in total darkness. Experiments of this type demonstrated the relationship between photopigment concentration and visual sensitivity and led to the photochemical theory of adaptation. It is apparent, however, that this theory can not account for all changes in visual sensitivity and that there must be some other mechanisms involved in

adaptation operating either in the photoreceptors or in more proximal regions of the retina (Boyton and Whitten, 1970; Grabowski et al., 1972; Werblin, 1974). These processes have been referred to as neural, or "network", adaptation. Neural mechanisms are more easily studied when background illumination is kept below a level which would bleach significant amounts of photopigment. Such processes operate not only in dark-adaptation but also in light-adaptation where small increases in ambient illumination lead to alterations in retinal sensitivity not dependent on photoreceptor mechanisms. Using the all-rod retina of the skate, Green et al. (1975) demonstrated that background light too weak to alter photoreceptor activity can decrease retinal sensitivity when measured in more proximal regions. Further studies have shown that in this species the retina apparently can be divided into two regions in which sensitivity control of the photoreceptors and horizontal cells can be separated from the activity of bipolar, amacrine and ganglion cells (Dowling and Ripps, 1976; Dowling and Ripps, 1977). Werblin and co-workers (Normann and Werblin, 1974; Werblin, 1974; Werblin and Copenhagen, 1974) have shown in Necturus that sensitivity control in each of three neuronal layers (photoreceptor, bipolar and ganglion cell) can be distinguished by varying the pattern of background light projected

on the retina. Such a separation of adaptation mechanisms occurring at different neuronal levels of the frog retina has yet to be demonstrated. In fact, Fusco and Hood (1974) could not demonstrate a difference between photoreceptor and ganglion cell responses in experiments involving changes in threshold with increases in background illumination. Gordon and Hood (1976), however, point out the limitations of increment threshold studies and indicate the importance of examining response vs stimulus intensity curves when studying adaptation mechanisms. The effect of weak background light on the response vs stimulus intensity curves was examined in the present study in order to determine whether or not adaptation processes independent of the photoreceptor response are present in the frog. Our experiments, using alterations in the aspartate-isolated mass receptor response as an index, demonstrated that background light at 0.016 fc reduces photoreceptor sensitivity while background light at 0.008 fc has no significant effect on photoreceptor activity. However, this weak background illumination (0.008 fc) reduces the sensitivity of neurons in the inner nuclear layer, as demonstrated by a shift of the b-wave R - log I curve to the right. These experiments clearly demonstrate that in the frog, as in skate and Necturus, adaptation mechanisms operating proximal to the pho-

toreceptors exist and that they can be elicited by weak background light. Furthermore, the idea that these adaptation mechanisms involve synaptic activity is supported by the fact that these alterations in sensitivity occur rapidly. Background light is maintained for only 4.5 seconds prior to stimulation in these experiments. This is sufficient time for neural adaptation processes to be elicited in Necturus (Copenhagen, 1975) and goldfish (Easter, 1968).

The weak background light used in these studies seems to affect primarily the rod system. This is supported by the fact that the implicit time of the AIMRR is altered only at stimulus intensities less than -2.0 log I. The faster response may be due to a sustained hyperpolarization of the rods brought about by the background illumination. This effect of background light on the rod vs cone contribution to the AIMRR is also manifested by the suppression of the biphasic waveform at stimulus intensities less than -2.0 log I (Fig. 11). This observation has also been reported in a recent study (Hemila, 1977).

Drug effects

GABA's presence in the retina has been known for 20 years. In studies of the retina's free amino acid content, GABA, together with taurine and glutamic acid, are always found to be in the highest concentrations (Kubicek and

Dolenek, 1958; Pasantes - Morales, 1972; Cohen et al., 1973). Noell (1959) was the first to show that exogenous GABA had an inhibitory effect on retinal neurons and subsequent studies involving the effect of GABA on individual neuron activity have supported this observation (Kishida and Naka, 1967, 1968; Straschill, 1968; Straschill and Perwein, 1969; Murakami et al., 1972). In the isolated superfused rabbit retina Ames and Pollen (1969) demonstrated that GABA at 10^{-4} M depressed both spontaneous and evoked activity of ganglion cells. The effect of exogenous GABA on field potentials, however, has been less clearly defined. Although Scholes and Roberts (1964) demonstrated that GABA depressed the chicken ERG at low stimulus intensity but enhanced it at high stimulus intensities, Pasantes - Morales et al. (1973) observed in the chicken a depression of the b-wave of the ERG at all stimulus intensities studied. Starr (1975b) demonstrated in the perfused rabbit eye cup preparation an increase in b-wave amplitude with GABA concentrations of 10^{-3} M, which was not blocked by 10^{-3} M bicuculline. However, picrotoxin, another GABA antagonist, abolished the ERG b-wave at 2×10^{-5} M. Bonaventure et al. (1974) similarly observed a depressant effect of picrotoxin at high concentrations on the ERG of the chicken. In the frog, Urban et al. (1976) observed that GABA (10^{-4} M) caused a depression

of the b-wave which could be antagonized by picrotoxin (5×10^{-4} M). These varying results might be the result of species differences or differences in stimulation parameters used to evoke the field potentials. Our studies in the frog demonstrate that GABA (10^{-4} M) depressed the b-wave of the ERG and that this depression could be reversed by picrotoxin (2×10^{-4} M). Furthermore, under scotopic conditions this depression could be observed at all stimulus intensities, from threshold to maximum evoked responses. GABA had no effect on the late receptor potential which supports an earlier observation (Jaffe et al., 1975). Picrotoxin administered alone increased b-wave amplitude possibly reflecting an antagonism of endogenous GABA. This is in opposition to the effect reported in rabbit (Starr, 1975b).

The inhibitory effect seen with GABA in the present study is in keeping with the inhibitory effects seen with this drug on practically all neurons in the vertebrate CNS (Krnjevic, 1974). Many studies have examined the effect of iontophoretically applied GABA, but in those in which neural tissue was perfused with media containing GABA the doses needed to evoke a response were comparable to those used in the present experiments. In the isolated frog spinal cord, GABA concentrations of 1 - 10 mM were needed to reduce the evoked ventral root response (Pixner, 1974), while in the

isolated rat spinal cord a depolarizing dorsal root potential was induced by GABA at 0.02 - 3.0 mM (Otsuka and Konishi, 1976). Nishi et al. (1974) observed a similar depolarization of primary afferent neurons in the bullfrog with GABA at concentrations of 0.01 - 0.1 mM. Furthermore, the influence of GABA on neuronal metabolism is evident only at higher concentrations. The stimulatory effect on ^{14}C - leucine incorporation into proteins of mitochondrial or ribosomal rat brain fractions by GABA is seen at concentrations (5.0 mM) 50 - 100 times higher than seen with ACh or norepinephrine (Baxter, 1976). Similarly, elevation of cyclic GMP levels in incubated slices of mouse cerebellum is seen only with GABA concentrations > 1.5 mM (Ferrendelli et al., 1974). The high concentration of amino acids needed to elicit a response when compared with cholinergic or catecholaminergic transmitters may in some way be related to their high levels within the CNS, which are approximately 3 orders of magnitude greater than ACh or the catecholamines (Krnjevic, 1974).

Since the b-wave is generated by Muller cells and is only secondarily an indication of neuronal activity, the possibility remains that the depression of b-wave amplitude by GABA is a direct action on these glial cells rather than an inhibition of neuronal activity. Krnjevic and Schwartz

(1967) observed that GABA could depolarize some glial cells in the cerebral cortex of the cat. This depolarizing effect is not associated with a conductance increase, however, and is thought to be related to an active uptake of GABA by these cells (Krnjevic, 1976). The fact that GABA is taken up solely by retinal neurons in the frog and not by Muller cells (Voaden et al., 1974) argues against this interpretation of GABA's effects in the present study. The observed effects of GABA and its antagonists on retinal field potentials appear to indicate that in the frog retina GABA is acting as an inhibitory transmitter. They do not, however, delineate any particular retinal function of this putative transmitter.

Previous studies have demonstrated a relationship between GABA levels and light- and dark-adaptation in the frog (Graham et al., 1970) and goldfish (Lam, 1972b) and it appears that the "GABA system" is more active under conditions of light. GABA neurons might act to decrease retinal sensitivity during light-adaptation. In keeping with this idea Graham (1974) reported that exogenous GABA raised, while picrotoxin and bicuculline decreased, b-wave threshold in the rat. Our studies also demonstrate that exogenous GABA can decrease sensitivity of the frog retina by shifting the b-wave R - log I curve to the right under scotopic con-

ditions. Its effect on light-adaptation mechanisms is not clear since it did not alter the extent to which weak background illumination decreased retinal sensitivity. The actions of picrotoxin in antagonizing endogenous GABA observed in these experiments might be more revealing. ERG responses in the presence of picrotoxin were larger in amplitude and slower in time-course, which is characteristic of dark-adapted responses. The b-wave R - log I curve is shifted to the left under scotopic conditions and the effect of weak background illumination is enhanced. These effects can be attributed to increased summation within the retina. In bleached retinas, where weak background light is not able to alter sensitivity, picrotoxin is still able to increase retinal sensitivity as evidenced by a shift in the b-wave R - log I curve to the left. It appears, therefore, that GABA behaves in a photomimetic manner while picrotoxin produces responses characteristic of dark-adaptation. Support for this idea is found in biochemical studies of the frog retina where rates of respiration were related to light stimulation and darkness. Oxygen consumption decreases in the light and this effect can be mimicked by the application of exogenous GABA (Jaffe et al., 1975). Picrotoxin had no effect on the late receptor potential indicating that observed alterations in the ERG must be dependent on mechanisms operating proximal

to the photoreceptors.

GABA neurons might be involved not only in control of retinal sensitivity but also in retinal functional organization. Wyatt and Daw (1976) have demonstrated that picrotoxin can destroy the directional sensitivity of rabbit ganglion cells, and Kirby and Enroth-Cugell (1976) have also reported changes in ganglion cell receptive fields in response to picrotoxin. Since GABA containing neurons have been demonstrated in association with both the inner (Kuriyama et al., 1968; Ehinger, 1970; Graham, 1972) and outer (Lam and Steinman, 1971; Yates and Keen, 1976) plexiform layers, it is possible that there are various subpopulations of GABA neurons subserving different functions.

The presence of glycine in the retina has been demonstrated in various species (Pasantes-Morales et al., 1972; Cohen et al., 1973; Yamamoto et al., 1970; Yates and Keen, 1976). Its candidacy as a possible retinal neurotransmitter is based on the demonstration of an active uptake mechanism for the amino acid (Bruun and Ehinger, 1972), of a light-evoked release (Bauer and Ehinger, 1977b) and of its inhibitory effect on retinal neurons (Ames and Pollen, 1969; Murakami et al., 1972). Our studies demonstrated a depressant effect on the ERG b-wave in frog similar to that seen with GABA. Glycine was less potent than GABA, however. A

concentration of 5×10^{-4} M was needed to cause a significant depression of the b-wave. This corresponds to what has previously been reported for isolated frog retina (Urban et al., 1976). Glycine had no effect on the aspartate-isolated mass receptor response which indicates that it must be acting at a site proximal to the receptors. Starr (1975b) reported similar results with glycine in rabbit. He observed a slight depression of b-wave amplitude with 1 mM glycine but no alteration of the a-wave. We have also shown that glycine desensitizes the retina, as evidenced by a shift in the R - log I curve to the right under scotopic conditions. It apparently has no effect, however, on adaptive mechanisms elicited by weak background light. Glycine's relationship to light- and dark-adaptation, therefore, is difficult to define.

The problem is further complicated by the fact that the depressant effect of glycine on the retina is not blocked by strychnine. In fact, strychnine *per se* causes a decrease in b-wave amplitude and virtually abolishes the ERG at a concentration of 1 mM. This is in keeping with the reported effects of high concentrations of strychnine in the isolated rabbit retina (Vorkel and Hanitzsch, 1971) but in contrast to observed effects in the *in situ* rabbit eye cup preparation (Starr, 1975b). The failure of strychnine to block glycine's

action in our experiments may indicate that glycine is not acting at the synaptic level in this preparation. Korol et al. (1975) reported a depression of the rabbit ERG after intravitreal injection of glycine, but they also observed cytopathological changes in amacrine cells. They suggested that glycine's action may be due to an overall membrane effect rather than interaction with specific synapses. This membrane effect has recently been questioned, but it was pointed out that exogenous glycine can effect a subpopulation of amacrine cells which do not take up this amino acid (Ehinger, 1977). Our results show that although both glycine and strychnine depress the b-wave of the ERG and shift its R - log I curve to the right, their actions are not identical. Glycine has no effect on the time-course of the b-wave, while strychnine significantly increases b-wave implicit time. This difference may point to an action of exogenous glycine which is not the same as that of the endogenously released compound, or it may indicate that strychnine is acting not simply as an antagonist of glycine. Strychnine has been shown to antagonize the actions of taurine (another inhibitory amino acid found in the retina) (Van Harreveld, 1976) and to have direct effects on neuronal membranes (Shapiro et al., 1974).

Malmfors (1963) reported a pronounced photophobia in

reserpinized animals and suggested that catecholamines might be mediating an inhibitory mechanism in normal retinas. It has been demonstrated that dopamine is the catecholamine present in retina (Haggendal and Malmfors, 1963; 1965) and that its levels can be influenced by states of light- and dark-adaptation (Drujan et al., 1965; Nichols et al., 1967; Drujan and Diaz Borges, 1968). For these reasons we attempted to relate dopamine to light- and dark-adaptation in the isolated frog retina. Exogenous dopamine at concentrations of 10^{-7} to 10^{-3} M had no effect, however, on the b-wave of the ERG. Neither the R - log I curve nor the implicit time of this response was altered. Similarly, dopamine had no effect on the photoreceptors as evidenced by a failure of this putative neurotransmitter to affect the amplitude or time-course of the aspartate-isolated mass receptor response. Application of dopamine has been shown to suppress ganglion cell activity in both rabbit (Ames and Pollen, 1969) and cat (Straschill and Perwein, 1969), but the data relating dopamine and retinal field potentials are equivocal. Graham (1974) reported that systemic reserpine caused no alterations in b-wave increment threshold curves in rats. Gutierrez and Spiguel (1971) observed no effect of reserpine on b-wave amplitude in cats. In rabbits, Hempel (1972) reported a suppression of b-wave amplitude with reserpine while Starr

(1975b) demonstrated a suppression of b-wave amplitude with dopamine. More recently, Ehinger and Nordenfelt (1977) produced a degeneration of dopaminergic retinal neurons in rabbit with 6-hydroxydopamine but observed no changes in the ERG. Our negative results in the frog retina may indicate that cells containing dopamine receptors lie proximal to those cells of the inner nuclear layer which contribute to the ERG b-wave, or alternatively that dopamine responding cells in the distal retina do not contribute significantly to field potentials generated in the distal retina.

Delayed off-response

The delayed off-response, or e-wave, like other components of the ERG probably reflects an algebraic summation of various potential changes occurring in the retina after light stimulation. It is possibly the result of rod-cone interaction (Crescitelli and Sickel, 1968; Chino and Sturr, 1975) and/or might be dependent on the interaction of excitatory and inhibitory processes occurring in more proximal layers of the retina (Pickering and Varju, 1971). The stage of adaptation (as measured by rhodopsin levels or the total time the retina is maintained in darkness) apparently does influence this delayed response (Crescitelli and Sickel, 1968; Pickering and Varju, 1971) and is the reason for the inclusion of this light-evoked potential in the present ex-

periments.

Our observations that GABA can affect the delayed off-response is not surprising in view of the relationship of GABA to light- and dark-adaptation reported previously (Graham et al., 1970; Lam, 1972; Graham and Pong, 1972). In the present study GABA increased and bicuculline decreased the amplitude of the e-wave. These results are in agreement with a photomimetic effect for increased GABA levels since Crescitelli and Sickel (1968) reported larger e-wave amplitudes with photopic responses than with scotopic responses. In their analysis of the delayed off-response, Pickering and Varju (1971) noted that the amplitude of the excitatory process elicited by an off-stimulus is dependent on the level of adaptation of the retina. It is conceivable, therefore, that changes in the e-wave are a reflection of GABA's role in the adaptation mechanism. The decrease in amplitude of the e-wave by bicuculline suggests the involvement of endogenous GABA in this delayed response since bicuculline can antagonize the actions of GABA (Curtis et al., 1970).

Although both GABA and bicuculline altered the amplitude of the e-wave, neither drug significantly changed the time - to - peak (TTP) of this response. Crescitelli and Sickel (1968) demonstrated that the amplitude and the delay of the e-wave are indications of different aspects of the

light history of the retina. Latency changes markedly only after short periods of bleaching and plateaus after longer bleaches. Rhodopsin levels have been shown to decrease rapidly with bleaching under these conditions, reaching 20% of dark-adapted levels after 5 minutes (Crescitelli and Sickel, 1968), while GABA levels in the retina show an increase during light stimulation with a much longer time-course (greater than one order of magnitude) (Lam, 1972b). It is suggested that the latency of the delayed off-response is dependent on receptor cell activity while the amplitude reflects more proximal neuronal activity involving GABA. This is supported by the fact that GABA and bicuculline alter e-wave amplitude without affecting latency. Conversely, the amplitude of the a-wave (a reflection of photoreceptor activity) is not altered by GABA or bicuculline. Finally, changes in wave length of stimulation alter the TTP of the delayed off-response without affecting its amplitude. A similar observation concerning GABA's role in frog retina has been reported by Burkhardt (1972). Picrotoxin was shown to increase amplitude of the proximal negative response (a measure of amacrine cell activity) without affecting its threshold or the stimulus intensity range over which changes in amplitude occur. It was suggested that GABA is acting primarily in proximal layers of the retina where summation

of neuronal activity would be more likely to affect amplitude of field potentials than threshold or latency.

The action of exogenous glycine seems to be a general depression of retinal response. Both b-wave and e-wave amplitude are depressed, and the establishment of a relationship between this putative transmitter and the delayed off-response requires more study. Dopamine, on the other hand, is without effect on the e-wave, which is consistent with its failure to alter other field potentials in the isolated frog retina.

SUMMARY

1 - Experiments were performed on the isolated retinas of frogs (Rana pipiens, southern) dark-adapted overnight. The preparation was superfused with Sickel's medium, oxygenated by bubbling air through it. Drugs were administered by dissolving them in the superfusing medium.

2 - The aspartate - isolated mass receptor response (AIMRR), the electroretinogram, and ganglion cell activity were examined under conditions in which the duration and intensity of the light stimulus and the level of background illumination were varied.

3 - Response versus stimulus intensity curves (R - log I) for the AIMRR and the ERG were generated under conditions of darkness and in the presence of background illumination. This permitted one to examine a) retinal sensitivity as the curve shifted on the log I scale and b) ceiling responses.

4 - Background light (0.008 fc) significantly shifts the R - log I curve for the b-wave to the right approximately 1 log unit. The R - log I curve for the AIMRR is unchanged.

5 - GABA (10^{-4} M) and glycine (5×10^{-4} M) significantly shift the b-wave R - log I curves to the right indicating decreased retinal sensitivity under scotopic conditions as well as after exposure to weak background illumination.

Neither putative neurotransmitter significantly affects the AIMRR.

6 - Picrotoxin, a GABA antagonist, reverses GABA depression and significantly shifts the b-wave R - log I curves to the left in both bleached and unbleached retinas, indicating increased retinal sensitivity. It is without effect on the AIMRR.

7 - Strychnine, a glycine antagonist, significantly shifts the b-wave R - log I curve to the right and is without effect on the AIMRR. In the presence of glycine depression, strychnine does not alter b-wave amplitude.

8 - Dopamine, (10^{-7} to 10^{-3} M), affects neither the b-wave of the ERG nor the AIMRR.

9 - The influence of background lighting in the presence and absence of drugs on the implicit time of the AIMRR and b-wave of the ERG was studied. AIMRR implicit time diminishes significantly in the presence of weak background light at stimulus intensities of -2.0 to -4.6 log I. On the other hand, b-wave implicit time lengthens with weak background illumination.

10 - Both picrotoxin and strychnine increase b-wave implicit time while GABA, glycine and dopamine are without effect on this parameter. None of these drugs alter the implicit time of the AIMRR.

11 - The delayed off-response, or e-wave, of the ERG was studied using varying intensities, durations and wavelengths of light stimulation. E-wave amplitude is inversely related to stimulus intensity and duration, while its time - to - peak (TTP) is directly related to these parameters. A red light stimulus (peaking at 650 nm) significantly shortened the TTP of the e-wave but did not alter the e-wave amplitude. Blue light stimuli (peaking at 400 nm) produced effects indistinguishable from white light stimuli. Neither a- nor b-wave amplitudes were significantly effected by changes in the wavelength of the stimulating light.

12 - GABA significantly increased e-wave amplitude in unbleached retinas without affecting TTP. This effect was significantly greater with red light stimulation than with white or blue. GABA did not alter the e-wave in the bleached retina.

13 - Bicuculline significantly decreases e-wave amplitude in unbleached retinas without affecting its TTP. In bleached retinas, picrotoxin decreases e-wave amplitude.

14 - Glycine depresses e-wave amplitude in unbleached retinas but is without effect on its implicit time.

15 - Dopamine (10^{-7} to 10^{-3} M) is without effect on e-wave amplitude or e-wave TTP.

16 - In microelectrode studies, ganglion cell spiking activ-

ity corresponded to the appearance of the e-wave. Spiking activity also coincided with the initiation of the b-wave.

17 - Of 25 ganglion cells (from 13 animals), 76% fired spontaneously, either slowly or with periodic bursts of activity. None of these responded to full field stimulation. Of the remaining cells, 3 were off-cells, 2 were on - off cells and 1 was an on-cell.

CONCLUSIONS

1 - In frogs (Rana pipiens, southern) adaptation mechanisms operating proximal to the photoreceptors exist and they can be elicited by weak background illumination.

2 - The contribution of rod and cone elements of the frog retina to light-evoked field potentials is modifiable by background illumination. Evidence for this is based on a) the conversion of the AIMRR from a biphasic to a monophasic waveform by background light, b) the conversion of the biphasic to a monophasic a-wave of the ERG by background light, and c) the alterations in implicit times of the AIMRR and the ERG b-wave by background illumination.

3 - Exogenous GABA depresses retinal sensitivity as the result of mechanisms operating in the retina proximal to the photoreceptors. The idea that endogenous GABA is involved in light-adaptation is supported by the fact that picrotoxin, a GABA antagonist, effectively reverses GABA depression and increases sensitivity in both bleached and unbleached retinas. Furthermore, the effectiveness of background light in decreasing retinal sensitivity is enhanced in the presence of picrotoxin. Exogenous GABA is also able to increase the amplitude of the ERG e-wave, a response associated with light- and dark-adaptation in the frog. The GABA antagonists

bicuculline and picrotoxin produce the opposite effect. These observations support the hypothesis that GABA plays a role in light-adaptation in the isolated frog retina.

4 - The role of glycine in neural adaptive mechanisms of the frog retina is unclear. The depression of retinal responsiveness to light stimulation occurs proximal to the photoreceptors affecting b-wave and e-wave amplitude and appears to be non-specific in nature in that it is not antagonized by strychnine and is without influence on retinal responsiveness to weak background illumination.

5 - It is unlikely that dopamine is involved in neural adaptation in the frog retina since it is without significant effect (over a wide range of concentrations) on retinal field potentials: b-wave amplitude, the R - log I curves of the b-wave and the AIMRR, e-wave amplitude and its implicit time (TTP).

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APPENDIX A

Intensities of the light stimulus were measured with a Hewlett-Packard radiant flux meter (Model 833 OA). With the probe placed in the position of the isolated retina preparation, stimulus intensity of the light source, both in the presence and absence of neutral density filters, was measured. Determinations of light intensity were made to the limit of sensitivity of the radiant flux meter. Measurements for white light stimuli and for those produced with broad band optical filters are given in the following table.

Color of light stimulus	log attenuation	mW/cm^2
White	0.0	13.35
	1.0	1.42
	2.0	0.192
	3.0	0.028
	4.2	0.0026
Red (650 nm)	0.0	11.10
	1.0	1.23
	2.0	0.160
	3.0	0.022
	4.2	0.0022
Blue (400 nm)	0.0	10.20
	1.0	1.08
	2.0	0.150
	3.0	0.022
	4.2	0.0016

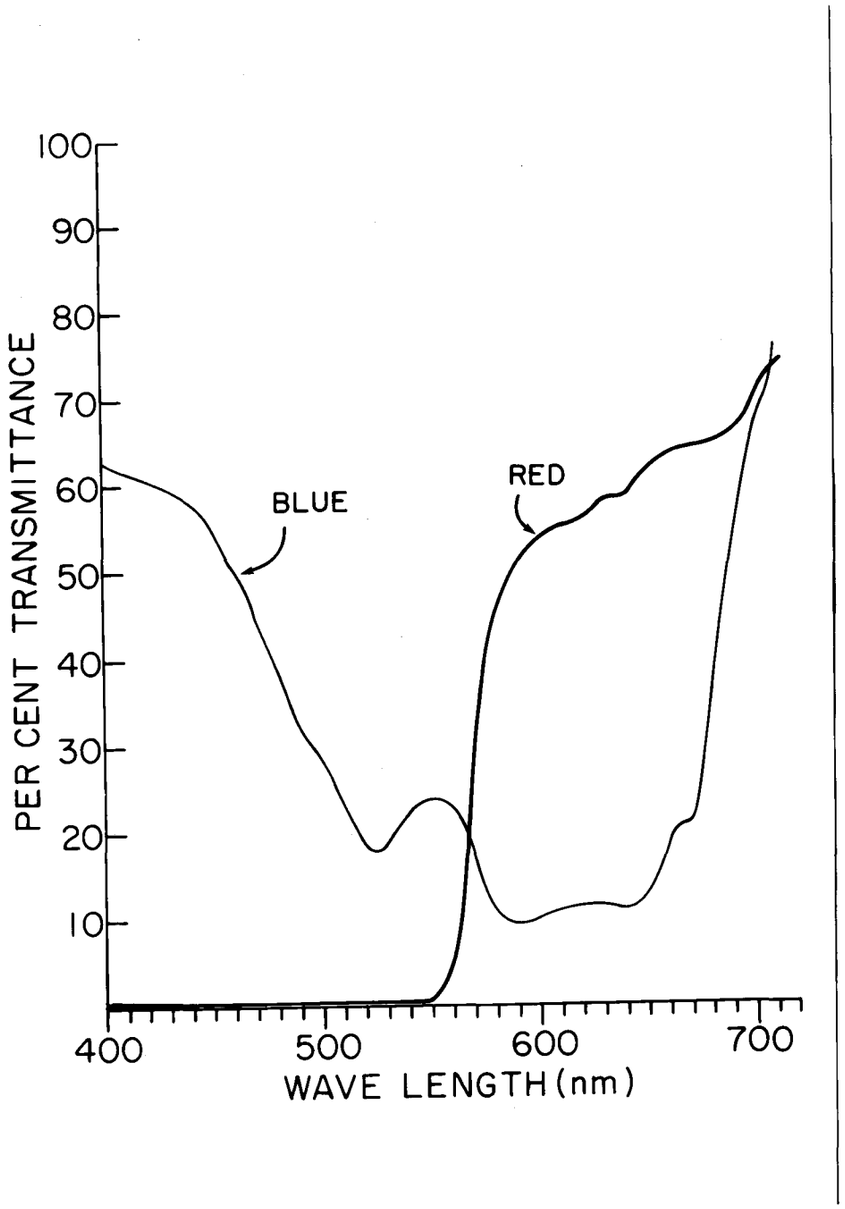

Fig. 46. Spectral transmittance of optical filters. "Blue" and "red" give the transmittance characteristics of the filters designated in the text blue (400 nm) and red (650 nm) respectively.

APPENDIX B

TABLE 1 EFFECT OF BACKGROUND LIGHT ON THE ASPARTATE-ISOLATED MASS RECEPTOR RESPONSE

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	100.0	98.3 ±1.2	96.6 ±2.1	96.1 ±2.1	81.9 ±4.7	81.6 ±1.5	60.6 ±5.3	50.9 ±1.8	27.7 ±2.7	19.4 ±3.1
Background light (0.008 fc)	93.0[b] ±5.8	86.6[b] ±6.6	86.3[b] ±8.2	84.3[b] ±9.0	73.0[b] ±9.2	70.0[b] ±6.9	54.0[b] ±8.3	47.4[b] ±4.1	22.6[b] ±1.5	15.1[b] ±1.6
Background light (0.016 fc)	60.8[a] ±5.4	64.6[a] ±7.5	58.4[a] ±5.2	56.1[a] ±5.1	45.8[a] ±6.6	44.2[a] ±3.2	29.8[a] ±6.5	19.4[a] ±0.4	5.4[a] ±2.1	1.9[a] ±1.9

Data expressed as mean per cent maximum response ± SEM.
n = 4
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.

[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 2 EFFECT OF BACKGROUND LIGHT (0.008 fc)
ON THE B-WAVE OF THE ERG

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	100.0	98.5 ±2.2	92.3 ±3.8	83.1 ±4.0	73.2 ±3.6	56.9 ±4.9	43.6 ±3.4	28.7 ±2.0	16.6 ±2.5	9.8 ±2.5
Background light (0.008 fc)	80.4[a] ±2.0	72.5[a] ±2.1	63.2[a] ±3.6	49.0[a] ±4.3	39.1[a] ±3.7	27.9[a] ±3.0	19.0[a] ±2.3	8.6[a] ±1.3	3.1[a] ±1.2	0.0[a]

Data expressed as mean per cent maximum response ± SEM.
n = 6
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.

TABLE 3 EFFECT OF BACKGROUND LIGHT ON THE IMPLICIT TIME OF THE ASPARTATE-ISOLATED MASS RECEPTOR RESPONSE

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control n=6	150.0 ±12.9	191.7 ±10.5	220.8 ±10.0	283.3 ±10.5	341.7 ±15.4	383.3 ±30.7	450.0 ±34.2	480.0 ±33.0	675.0 ±47.9	
Background light (0.008 fc) n=6	137.5[b] ±12.5	170.0[b] ±12.2	195.0[b] ±20.0	265.0[b] ±15.0	291.7[a] ±15.4	333.3[a] ±21.1	375.0[a] ±17.1	404.2[a] ±13.6	500.0[a] ±40.8	
Control n=4	112.5 ±12.5	133.3 ±16.7	162.5 ±23.9	183.3 ±33.3	237.5 ±42.7	233.3 ±33.3	293.8 ±38.7	316.7 ±16.7	475.0 ±47.9	733.3 ±66.7
Background light (0.016 fc) n=4	112.5[b] ±12.5	133.3[b] ±16.7	150.0[b] ±20.4	166.7[b] ±16.7	200.0[b] ±35.4	200.0[b] ± 0.0	225.0[a] ±25.0	241.7[a] ±8.3	283.3[a] ±16.7	316.7[a] ±16.7

Data expressed as mean implicit time (msec) ± SEM.
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 4 EFFECT OF BACKGROUND LIGHT ON THE IMPLICIT TIME OF THE B-WAVE OF THE ERG

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	284.1 ±21.9	297.9 ±19.1	318.8 ±19.5	341.7 ±21.0	358.3 ±25.1	404.2 ±28.3	439.6 ±26.7	520.8 ±34.9	595.8 ±46.6	565.0 ±42.7
Background light (0.008 fc)	329.2[b] ±34.5	352.1[b] ±38.2	379.5[a] ±36.0	435.4[a] ±33.2	464.6[a] ±32.8	487.5[a] ±31.3	514.6[a] ±26.9	540.9[b] ±33.4	537.5[b] ±36.0	533.3[b] ±44.1

Data expressed as mean implicit time (msec) ± SEM.
n = 12
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 5 EFFECT OF GABA (10^{-4} M)
ON B-WAVE R - LOG I CURVES

	Illumination Intensity (log I)						
	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0	-7.0
Dark control	100.0	98.6 ±2.4	92.2 ±3.2	68.4 ±5.9	42.6 ±6.2	17.8 ±4.8	3.6 ±2.8
Background light (0.008 fc)	64.0[a] ±7.1	53.6[a] ±7.0	47.7[a] ±4.4	28.1[a] ±3.5	10.0[a] ±3.3	3.3[b] ±2.2	0.0
Dark + GABA	67.1[a] ±5.9	67.8[a] ±4.3	62.7[a] ±5.8	43.3[a] ±5.8	25.2[a] ±4.7	10.8[b] ±3.8	0.0
Background light (0.008 fc) + GABA	40.0[c] ±5.0	36.5[c] ±3.0	30.3[c] ±2.6	20.0[c] ±2.4	5.5[c] ±3.3	1.2 ±1.3	0.0

Data expressed as mean per cent maximum response ± SEM.
n = 7
a Statistically significant difference from control ($p < 0.05$); paired Student t-test.
b Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.
c Statistically significant difference from dark + GABA ($p < 0.05$); paired Student t-test.

TABLE 6 EFFECT OF GABA (10^{-4} M) ON THE ASPARTATE-ISOLATED MASS RECEPTOR RESPONSE

	Illumination Intensity (log I)						
	0.0	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0
Control	100.0	96.0 ±2.4	82.7 ±3.1	69.8 ±1.6	48.9 ±4.8	16.1 ±2.8	0.0
GABA	103.2[a] ±3.2	103.9[a] ±3.6	87.2[a] ±4.4	70.7[a] ±3.2	51.8[a] ±4.4	19.8[a] ±5.7	0.0

Data expressed as mean per cent maximum response ± SEM.
n = 4
[a] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 7 EFFECT OF PICROTOXIN (10^{-4} M)
ON B-WAVE R - LOG I CURVES

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Dark control	100.0	97.3 ±1.8	90.9 ±2.1	87.4 ±5.0	79.9 ±5.5	67.8 ±6.6	60.6 ±6.7	41.1 ±5.4	27.2 ±5.2	19.5 ±4.5
Background light (0.008 fc)	76.6[a] ±4.0	68.3[a] ±4.3	61.4[a] ±2.7	54.2[a] ±2.1	48.9[a] ±2.6	40.4[a] ±3.0	35.0[a] ±3.5	22.0[a] ±3.7	12.8[a] ±4.1	5.0[a] ±2.6
Dark + Picro.	122.6[b] ±12.6	115.4[b] ±10.2	120.9[b] ±14.5	118.1[a] ±14.8	114.0[a] ±16.1	106.0[a] ±16.4	96.8[a] ±15.0	77.8[a] ±11.8	63.1[a] ±12.4	46.2[a] ±13.2
Background light (0.008 fc) + Picro.	78.2 ±12.8	71.3 ±7.1	63.3 ±5.8	55.7 ±6.7	51.5 ±6.1	44.9 ±7.7	40.7 ±7.2	29.7 ±8.6	15.8 ±6.3	9.4 ±4.2

Data expressed as mean per cent maximum response ± SEM.
n = 6
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 8 EFFECT OF PICROTOXIN (10^{-4} M) ON B-WAVE R - LOG I CURVES OF LIGHT-ADAPTED ANIMALS

	Illumination Intensity (log I)						
	0.0	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0
Dark control	100.0	97.7 ±2.3	89.1 ±6.4	74.9 ±8.3	40.1 ±4.1	13.4 ±1.4	0.0
Background light (0.008 fc)	100.0	97.0[b] ±3.0	86.2[b] ±5.4	70.1[b] ±7.9	39.2[b] ±5.9	11.3[b] ±0.4	0.0
Dark + Picro.		128.3[a] ±2.7	113.6[a] ±2.4	101.7[a] ±4.1	69.0[a] ±1.1	20.6[a] ±1.7	11.5[a] ±1.3
Background light (0.008 fc) + Picro.		124.7 ±4.8	113.8 ±1.7	97.5 ±0.7	67.4 ±3.7	25.4 ±3.2	9.1 ±1.1

Data expressed as mean per cent maximum response ± SEM.
n = 3
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 9 EFFECT OF PICROTOXIN (10^{-4} M) ON THE ASPARTATE-ISOLATED MASS RECEPTOR RESPONSE

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	100.0	92.9 ±2.8	86.8 ±3.6	74.0 ±3.1	65.6 ±3.3	51.0 ±2.8	35.0 ±2.1	20.5 ±2.6	13.4 ±3.1	6.7 ±2.3
Picrotoxin	93.9[a] ±4.7	89.6[a] ±4.3	82.7[a] ±4.1	71.4[a] ±4.4	61.7[a] ±4.3	49.4[a] ±3.0	38.7[a] ±2.4	23.0[a] ±3.4	13.5[a] ±3.6	8.0[a] ±2.5

Data expressed as mean per cent maximum response ± SEM.
n = 6
[a] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 10 EFFECT OF GLYCINE ($5x10^{-4}$ M)
ON B-WAVE R - LOG I CURVES

	Illumination Intensity (log I)						
	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0	-7.0
Control	100.0	96.4 ±5.1	92.8 ±6.5	69.1 ±7.9	42.5 ±5.8	17.1 ±2.2	0.0
Background light (0.008 fc)	69.5[a] ±5.8	63.3[a] ±5.8	50.6[a] ±6.5	38.7[a] ±6.8	17.2[a] ±5.9	6.9[b] ±4.2	0.0
Dark + glycine	53.2[a] ±4.6	51.6[a] ±5.1	49.1[a] ±4.7	30.1[a] ±4.4	16.9[a] ±2.8	7.1[a] ±0.7	0.0
Background light (0.008 fc) + gly.	30.0[c] ±3.5	29.9[c] ±4.2	26.8[c] ±2.4	16.2[c] ±1.6	3.2[c] ±1.4	1.0[c] ±1.0	0.0

Data expressed as mean per cent maximum response ± SEM.
n = 5
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.
[c] Statistically significant difference from dark + glycine ($p < 0.05$); paired Student t-test.

TABLE 11 EFFECT OF STRYCHNINE (10^{-4} M)
ON B-WAVE R - LOG I CURVES

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	100.0	96.2 ±0.8	96.1 ±1.5	89.7 ±2.8	88.2 ±5.2	81.8 ±5.3	70.9 ±5.2	59.4 ±4.2	40.1 ±4.9	31.7 ±4.7
Background light (0.008 fc)	80.9[a] ±4.3	73.5[a] ±3.9	69.4[a] ±4.7	60.2[a] ±4.8	57.0[a] ±6.5	49.3[a] ±6.4	42.1[a] ±5.7	35.0[a] ±5.3	22.2[a] ±3.0	10.8[a] ±2.7
Dark + strych.	51.3[a] ±4.5	48.1[a] ±4.5	45.5[a] ±4.4	38.5[a] ±3.6	35.3[a] ±3.4	31.2[a] ±3.6	28.2[a] ±3.5	21.6[a] ±3.8	12.9[a] ±3.7	10.9[a] ±4.4
Background light (0.008 fc) + strych.	38.9[b] ±4.4	35.9[b] ±3.8	32.1[b] ±4.4	28.8[b] ±5.3	24.8[b] ±4.9	20.0[b] ±4.3	14.8[b] ±2.9	7.6[b] ±3.1	1.8[b] ±1.8	1.5 ±1.5

data expressed as mean per cent maximum response ± SEM.
n = 6
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Statistically significant difference from dark + strych. ($p < 0.05$); paired Student t-test.

TABLE 12 EFFECT OF STRYCHNINE (10^{-4} M)
ON B-WAVE IMPLICIT TIME

	Illumination Intensity (log I)									
	0.0	-0.6	-1.0	-1.6	-2.0	-2.6	-3.0	-3.6	-4.2	-4.6
Control	275.0 ±41.1	290.0 ±35.9	295.0 ±33.9	295.0 ±33.9	340.0 ±48.5	355.0 ±22.9	385.0 ±15.0	415.0 ±10.0	480.0 ±25.5	550.0 ±31.6
Strychnine	370.0[a] ±28.9	430.0[a] ±46.4	465.0[a] ±60.0	505.0[a] ±42.1	550.0[a] ±35.3	580.0[a] ±46.4	640.0[a] ±57.9	705.0[a] ±66.3	820.0[a] ±111.4	912.5[b] ±171.2

Data expressed as mean implicit time (msec) ± SEM.
n = 5
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 13 EFFECT OF DOPAMINE (10^{-4} M)
ON B-WAVE R - LOG I CURVES

	Illumination Intensity (log I)						
	-1.0	-2.0	-3.0	-4.0	-5.0	-6.0	-7.0
Control	100.0	91.8 ±2.6	82.8 ±2.8	59.0 ±2.1	31.0 ±3.9	14.8 ±1.6	0.0
Background light (0.008 fc)	80.5 ±0.9	71.6 ±1.2	55.5 ±2.0	38.4 ±1.6	17.7 ±2.4	9.4 ±1.5	0.0
Dark + dopamine	110.6[a] ±15.7	100.9[a] ±12.7	86.4[a] ±9.7	53.3[a] ±4.5	30.8[a] ±1.7	15.7[a] ±0.6	0.0
Background light (0.008 fc) + dop.	94.4 ±15.3	81.0 ±11.8	62.3 ±10.4	36.7 ±3.4	17.5 ±1.6	9.5 ±2.1	0.0

Data expressed as mean per cent maximum response ± SEM.
n = 4
[a] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 14 EFFECT OF STIMULUS WAVELENGTH ON ERG COMPONENTS OF ISOLATED FROG RETINA

Color of light stimulus	Stimulus duration (msec)	n	a-wave amplitude (μV ± S.E.M.)	b-wave amplitude (μV ± S.E.M.)	e-wave amplitude (μV ± S.E.M.)	TTP e-wave (sec ± S.E.M.)
White	200	8	67.2 ± 9.6	162.7 ± 17.2	32.4 ± 6.3	9.6 ±0.8
Blue (400 nm)	200	8	76.2 ±12.0	186.7 ± 32.5	35.1 ± 8.6	9.7 ±0.6
Red (650 nm)	200	8	65.5 ±10.5	175.2 ± 35.2	32.2 ± 7.4	7.0 ±0.6*

* Indicates these data differ significantly from those obtained for blue or white light stimuli ($p < 0.05$).

TABLE 15 EFFECT OF GABA (10^{-4} M) ON ERG COMPONENTS OF ISOLATED FROG RETINA

Color of light stimulus	a-wave amplitude		b-wave amplitude		e-wave amplitude		TTP e-wave	
	cont.	GABA	cont.	GABA	cont.	GABA	cont.	GABA
White	100.0 ±5.8	102.9[b] ±3.5	100.0 ±4.8	76.4[a] ±3.6	100.0 ±7.2	120.2[a] ±11.4	100.0 ±3.8	103.1[b] ±2.4
Blue (400 nm)	100.0 ±5.0	106.2[b] ±2.5	100.0 ±4.2	71.2[a] ±3.5	100.0 ±10.5	140.6[a] ±10.0	100.0 ±2.3	105.1[b] ±6.2
Red (650 nm)	100.0 ±3.8	98.9[b] ±1.8	100.0 ±7.0	83.0[a] ±2.5	100.0 ±7.2	154.0[a] ±11.2	100.0 ±2.3	106.8[b] ±2.0

Data expressed as mean per cent of control ± SEM.
n = 8
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

TABLE 16 EFFECT OF BICUCULLINE (2×10^{-4} M)
ON ERG COMPONENTS OF ISOLATED FROG RETINA

Color of light stimulus	a-wave amplitude		b-wave amplitude		e-wave amplitude		TTP e-wave	
	cont.	Bicuc.	cont.	Bicuc.	cont.	Bicuc.	cont.	Bicuc.
White	100.0 ±11.1	96.7[b] ±4.1	100.0 ±6.5	93.7[b] ±1.8	100.0 ±8.4	75.7[a] ±3.6	100.0 ±6.8	101.5[b] ±2.3
Blue (400 nm)	100.0 ±7.4	97.0[b] ±2.4	100.0 ±2.2	88.2[a] ±3.6	100.0 ±5.5	74.9[a] ±4.0	100.0 ±8.2	100.0[b] ±2.8
Red (650 nm)	100.0 ±4.5	97.8[b] ±2.4	100.0 ±4.7	91.4[b] ±2.3	100.0 ±5.1	70.6[a] ±4.6	100.0 ±6.3	101.2[b] ±3.3

Data expressed as mean per cent of control ± SEM.
n = 8
[a] Statistically significant difference from control ($p < 0.05$); paired Student t-test.
[b] Not a statistically significant difference from control ($p > 0.05$); paired Student t-test.

APPENDIX C

TABLE 17 SURVEY OF DRUGS

Drug	conc. µM	ERG a-wave amplitude	ERG b-wave amplitude	AIMRR amplitude
Norepinephrine	1	0	0	
	10	0	0	
Acetylcholine	1	0	0	
	10	0	0	
	1000	-	-	
Physostigmine	10	0	0	
Serotonin	1	0	-	
	10	++	++	
Melatonin	10	0	+	
Taurine	10	0	+	
	100	0	++	
	1000	0	--	
Histamine	1	0	-	
	10	++	+++	
Histidine	10	+	++	
Phenobarbital	1000	-	+++	
	5000	---	---	
Haloperidol	10	0	0	
3;5'-cyclic AMP	1			-
	10			+
3;5'-cyclic GMP	10			0

0 = no change

+ = 0-20% ↑ - = 0-20% ↓
++ = 20 - 50% ↑ -- = 20-50% ↓
+++ = 50 - 100% ↑ --- = 50-100% ↓

APPENDIX D

The relationship between the AIMRR implicit time and stimulus intensity can be described by a curve consisting of two distinct components. Student's t - test was used to determine whether or not the slopes of these components differ significantly from each other. Curvilinear regression lines were fitted to the data by the method of "least squares fit" and the estimates of slope (b) were derived by a computer program. Slope components of implicit time vs stimulus intensity curves were determined for curves generated in the dark (Part I) and in the presence of background light (Part II). Components of the curves used in the following summary of calculations are given in Fig. 47.

Part I: AIMRR implicit times in the dark.

$$b_1 = A_0 - A_1 = 163.95$$
$$b_2 = A_1 - A_2 = 59.75$$
$$b_t = A_0 - A_2 = 104.00$$

$$t = \frac{b_1 - b_2}{S_{y.x}\sqrt{1/SS_{x1} + 1/SS_{x2}}} = \frac{163.95 - 59.75}{S_{y.x}\sqrt{1/28.76 + 1/245.2}}$$

$$t = \frac{104.20}{S_{y.x}\ (0.1971)}$$

$$S_{y.x} = \sqrt{\frac{SS_1 \text{ dev. regression} + SS_2 \text{ dev. regression}}{df_1 \text{ dev. regression} + df_2 \text{ dev. regression}}}$$

$$S_{y.x} = \sqrt{\frac{238471.36 + 40943.46}{19 - 2 + 16 - 2}} = \sqrt{\frac{279414.8}{31}} = 94.94$$

$$t = \frac{104.20}{94.94\ (0.1971)} = \frac{104.20}{18.71} = 5.5659 \qquad p < 0.01$$

Slopes are significantly different.

Part II: AIMRR implicit times in the presence of background light.

$b_1 = B_0 - B_1 = 41.75$
$b_2 = B_1 - B_2 = 44.68$
$b_t^2 = B_0 - B_2 = 41.34$

$$t = \frac{b_1 - b_2}{S_{y.x}\ \sqrt{1/SS_{x1} + 1/SS_{x2}}} = \frac{2.926}{S_{y.x}\ \sqrt{1/28.76 + 1/227.56}}$$

$$t = \frac{2.926}{S_{y.x}\ (0.1979)}$$

$$S_{y.x} = \sqrt{\frac{SS_1 \text{ dev. regression} + SS_2 \text{ dev. regression}}{df_1 \text{ dev. regression} + df_2 \text{ dev. regression}}}$$

$$S_{y.x} = \sqrt{\frac{25729.1 + 29032.4}{16 - 2 + 18 - 2}} = \sqrt{\frac{54761.5}{30}} = 42.72$$

$$t = \frac{2.926}{42.72\ (0.1979)} = \frac{2.926}{8.456} = 0.3461$$

Slopes are not significantly different.

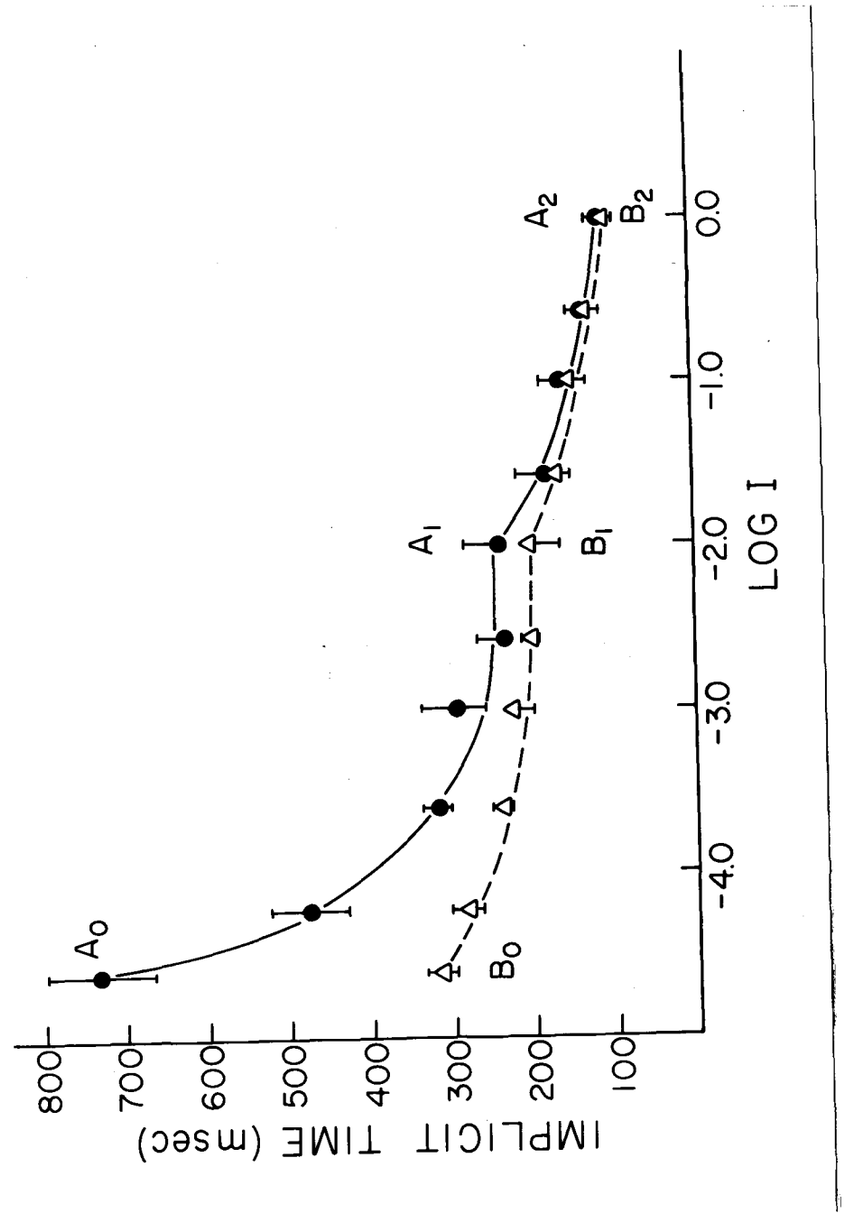

Fig. 47. Slope components of AIMRR implicit time. Solid circles ● indicate AIMRR implicit time in the dark. Open triangles Δ indicate AIMRR implicit time in the presence of background light (0.016 fc).

APPROVAL SHEET

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

7 December 1978
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