

EFFECT OF BACKGROUND ILLUMINATION
ON
HORIZONTAL CELL RECEPTIVE-FIELD SIZE
IN
THE RETINA OF THE GOLDFISH
(*CARASSIUS AURATUS*)

By

WILLIAM HAROLD BALDRIDGE, B.SC.

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EFFECT OF BACKGROUND LIGHT ON GOLDFISH HORIZONTAL CELLS

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AUTHOR: William Harold Baldrige,
B.Sc. (Univ. of Toronto)

SUPERVISOR: Professor A.K. Ball

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ABSTRACT

The mechanisms underlying light-adaptation in goldfish retinal horizontal cells were investigated. The receptive-field size of horizontal cells was reduced by background illumination, but by a mechanism not mediated by dopamine, the only known modulator of horizontal cell receptive-field size. Light-induced changes in receptive-field size were shown to vary depending on which of two adaptation states the retina was in prior to background illumination. Presentation of background illumination to a dark-adapted retina resulted in a light-sensitized retina in which horizontal cell receptive-field size and responsiveness was increased. Application of background illumination to light-sensitized retinas lead to light-adapted retinas, in which horizontal cell receptive-field size was decreased.

Two new putative adrenergic neurons were identified immunohistochemically in the goldfish retina. These cells, in particular a new type of interplexiform cell that could provide direct synaptic input to horizontal cells, raised the possibility that the light-dependent reduction in horizontal cell receptive-field size might be due to the release of an adrenergic transmitter. However, studies showed that the effects of adrenergic transmitters on horizontal cells were more consistent with an action at dopamine receptors than at

adrenoreceptors. Because it was previously shown that the light-dependent reduction in horizontal cell receptive-field size did not depend on the action of dopamine, it was concluded that the adrenergic neurons are unlikely to be involved in this change.

Studies on the effects of nitric oxide suggest that this free radical gas may be involved in the background illumination-induced reduction of horizontal cell receptive-field size. This was demonstrated by showing that horizontal cells are immunoreactive for nitric oxide synthase and that two inhibitors of nitric oxide synthase reduced the effects of background illumination on horizontal cell receptive-field size.

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LIST OF ABBREVIATIONS

λ	space constant
[³ H]-	tritium
6-OHDA	6-hydroxydopamine
5-HT	5-hydroxytryptamine (serotonin)
APB	L-2-amino-4-phosphonobutyrate
APTEX	3-amino-propyltriethoxysilane
BC	bipolar cell
cyclic AMP	cyclic 3',5'-adenosine monophosphate
cyclic GMP	cyclic 3',5'-guanosine monophosphate
DA	dopamine
DBH	dopamine- β -hydroxylase
EGTA	ethylene glycol-bis(β -aminoethyl ether) N,N,N',N'-tetraacetic acid
EPI	epinephrine
FRMFamide	molluscan cardioexcitatory peptide
GABA	γ -aminobutyric acid
GAD	glutamic acid decarboxylase
GC	ganglion cell
GLU	glutamate
GnRH	gonadotropin-releasing hormone
HAT	horizontal cell axon terminal
HC	horizontal cell
HPLC	high pressure liquid chromatography

INL	inner nuclear layer
IPL	inner plexiform layer
IR	immunoreactive
L-ARG	L-arginine
L-NAME	N ^o -nitro-L-arginine methyl ester
L-NOLA	N _o -nitro-L-arginine
NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
OPL	outer plexiform layer
PBS	phosphate buffered saline
PC	photoreceptor
PDA	(±)- <i>cis</i> -2,3-piperidinedicarboxylic acid
PKC	protein kinase C
PNMT	phenylethanolamine-N-methyltransferase
R _i	gap junction resistance
R _m	membrane resistance
RB	Rinse buffer
RPE	retinal pigmented epithelium
TH	tyrosine hydroxylase

I. INTRODUCTION

Since Ramón y Cajal's (1892) classical description, much has been learned about the anatomy and physiology of the vertebrate retina (Dowling 1987). In addition to the desire to understand the basic mechanisms of vision, an interest in how the retina can operate in so many light environments has motivated vision scientists for decades.

At the forefront of these investigations has been the study of the teleost retina, and in particular, the retinas from the family of fishes called Cyprinidae. These animals are familiarly known as carps and goldfish. One reason that the study of fish retina is so compelling is that it shares many similarities with the human visual system. Unlike many laboratory animals, which are typically nocturnal, fish have a visual system that is active in both day and night and have a sensitivity to colour similar to man (Brown and Wald 1963, 1964; Marks 1965).

The retina is capable of adjusting to changes in ambient illumination by processes which are intrinsic to the photoreceptors as well as in the pathways used for information processing. The latter changes are termed network adaptation (Dowling 1987). Network adaptation is revealed by changes in the receptive-field properties of sustained ganglion cells which are integral to "form" vision. The receptive fields of

ganglion cells result from interactions between photoreceptors, bipolar cells, horizontal cells and amacrine cells. Changes in the properties of sustained ganglion cells during adaptation reflect changes in the activity of these other retinal neurons.

The purpose of this thesis is to elucidate some of the mechanisms underlying changes in the properties of horizontal cells due to changes in the adaptation state of the retina. The purpose of this introduction is to review the basic anatomy and physiology of the vertebrate retina in order to describe how sustained ganglion cell receptive fields are formed (Section 2.0). The structural and functional changes of the retina associated with adaptation will be reviewed emphasizing how horizontal cell activity alters ganglion cell receptive fields (Section 3.0). The specific aims of the experimental work will be presented (Section 4.0), followed by my studies of the effect of light-adaptation on horizontal cells in the goldfish retina (Chapters II - VI). Finally, the thesis shall be concluded by a discussion of the significance of the work (Chapter VI).

1.0 The retina

The retina is a thin sheet of nervous and epithelial tissue at the back of the eye. According to Polyak (1957) the first accurate description of the retinal layers was made by Müller (1856). Ramón y Cajal (1892) listed ten layers of the retina which he based on the observations of Schwalbe (1874)

and Ranvier (1875). Ramón y Cajal (1892) gave the name "plexiform" to the layers of the retina previously called granular, molecular, reticular, and a variety of other descriptions. Modern terminology (Polyak 1957) groups the somata of horizontal cells, bipolar cells and amacrine cells, which Ramón y Cajal considered as separate layers, together as the inner nuclear layer. The layers of the retina are summarized in Table 1.

In addition to the description of retinal anatomy by layer, other descriptions in the literature are common. The retina is sometimes divided into two parts, the outer and inner retina (see Table 1). Bipolar cells and the "special stellate cells" of Ramón y Cajal (1892), later called "interplexiform cells," (Gallego 1971) traverse the inner and outer retina border. The landmark for direction in the retina is the ganglion cell layer. The direction away from the ganglion cell layer toward the photoreceptors is said to be distal whereas the direction toward the ganglion cell layer is proximal (Table 1).

2.0 Neurons of the retina and form vision

2.1 Photoreceptors

The nuclei of photoreceptors lie in the outer nuclear layer and their synaptic terminals are located in the outer plexiform layer. Photoreceptor outer segments, known to be the light-sensitive elements since the late nineteenth century (Kühne 1878; Boll 1887), lie in the visual cell layer of the

Table 1.--Layers and orientation within the retina

	LAYER NAME	CELLS IN LAYER	
1.	epithelial or pigment layer	retinal pigmented epithelium	(distal) OUTER RETINA
2.	visual cell layer	outer segments of rods and cones	
3.	outer nuclear layer	nuclei of rods and cones	
4.	outer plexiform layer	rod and cone terminals and horizontal and bipolar cell dendrites	
5.	inner nuclear layer	horizontal cells	
6.		bipolar cells	(proximal) INNER RETINA
7.		amacrine cells	
8.	inner plexiform layer	axon terminals of bipolar cells, amacrine cell and ganglion cell processes	
9.	ganglion cell layer	ganglion cells	
10.	optic nerve fibre layer	axons of ganglion cells	

Modified from Ramón y Cajal (1892) and Polyak (1957).

retina and are separated from the inner segment (containing the nucleus and synaptic terminal) by the outer limiting membrane.

Two types of photoreceptor cells, the cones and the rods, were first distinguished on morphological grounds by Schultze (1866). Schultze (1866) noted that the retinas of nocturnal animals were dominated by rods and proposed that rods are important during vision at night (scotopic vision) whereas cones function during daylight (photopic vision).

2.2 Photoreceptor physiology

The first recordings from cone photoreceptors were made in the carp retina (Tomita 1965). The cells had membrane potentials in the dark of -30 to -40 mV, hyperpolarized to light and could be classified into three types based on the maximum sensitivity at three wavelengths of light: red (625 nm), green (530 nm) or blue (455 nm). This finding corresponded to the three types of photopigment identified by microspectrophotometry in goldfish cones (Marks 1965). The first recordings from rods were from frog and, like cones, hyperpolarized to light (Toyoda et al. 1970). More detailed studies in the mudpuppy confirmed the Duplicity Theory of Schultze (1866) by showing the sensitivity of rods was greater (100 to 1000 times) than cones (Fain and Dowling 1973). The maximum spectral sensitivity of the rod response was near 500 nm, in agreement with microspectrophotometric measurements of mammalian (Brown and Wald 1964) and carp (Hanaoka and Fujimoto

1957) rods. It has not been possible to obtain intracellular recordings from teleost rods because of their small size ($< 1 \mu\text{m}$).

2.3 Why photoreceptors hyperpolarize to light

The mechanism of phototransduction has been intensively studied. The dark standing current of photoreceptors is generated by the inward movement of cations, predominantly Na^+ , across the outer segment membrane (Hagins, Penn and Yoshikami 1970). The dark current results in the photoreceptor membrane being depolarized. Photoisomerization of rhodopsin results in an intracellular cascade that lowers cyclic GMP levels (Miki et al. 1973; Woodruff and Bownds 1979; Fung and Stryer 1980; Kühn et al. 1981; Schoenlein et al. 1991). Since cyclic GMP has been shown to open directly cation channels in bovine rods (Fesenko, Kolenikov and Lyuborsky 1985) and catfish cone outer segments (Haynes and Yau 1985), in the dark the photoreceptor is depolarized. In the light a reduction in cyclic GMP results in a hyperpolarization of the photoreceptor.

Threshold measurements have shown that the rod photoreceptors are more sensitive to light than cones (Fain and Dowling 1973). Although the different photopigments permit the cones to be maximally sensitivity to different wavelength of light, there do not appear to be any marked sensitivity differences between the photopigments. The increased sensitivity of rods is explained partly by the

larger cross-sectional area of the rod outer segments, but there is also an inherently greater gain (Fain and Dowling 1973). This suggests that a single photon produces a greater cascade of events at some unknown point in phototransduction in rods compared to cones.

2.4 Horizontal cells

2.5 Horizontal cell types

Horizontal cells were first identified in the retina of carp and other teleosts by Ramón y Cajal (1892). He differentiated three types of horizontal cells (outer, middle, and inner) according to their location in the retina. Horizontal cell somata are located in the outer nuclear layer and extend dendrites into the outer plexiform layer. The dendritic field of each horizontal cell is about 100 μm . Ramón y Cajal also believed that each cell had a long thin axon that extended proximally (outer and intermediate cells) or distally (inner cells) but the termination of these processes could not be identified. With great foresight Ramón y Cajal suggested that horizontal cells "are for the mediation of lateral associative connections of the visual cells" (Ramón y Cajal 1892).

Some sixty years later teleost horizontal cells were the first neurons of the vertebrate retina from which intracellular recordings were made (Svaetichin 1953, 1956). The cells had membrane potentials of about -30 mV in the dark and responded to light with graded changes in potential. Two

types of responses were identified. Some cells hyperpolarized to light regardless of wavelength. These cells were called luminosity type (L-type). Two other types of cells depolarized to either red or blue wavelengths, respectively, and hyperpolarized to all other wavelengths. These cells were called chromaticity type (C-type) (Svaetichin 1956). Because the location of the electrode could not be verified, it was believed that the recordings came from cone photoreceptors (Svaetichin 1953, 1956). Intracellular staining, using crystal violet, later showed that these responses came from the horizontal cell layers described by Ramón y Cajal (1892). C-type responses came from horizontal cells more proximally located than L-type responses (MacNichol and Svaetichin 1958; Svaetichin and MacNichol 1958).

Using early techniques to stain cells recorded by intracellular microelectrodes, Mitarai and his colleagues characterized further horizontal cells and identified four types of responses in carp retina. In addition to the one L-type and two C-type responses identified previously, a fourth L-type response showed greater sensitivity (Mitarai and Yagasaki 1955) and had a rod-like spectral sensitivity (Mitarai 1964, 1965). It was suggested that this scotopic L-type response was due to rod input. The scotopic L-type response came from horizontal cells that were located proximally to the horizontal cells that produced photopic L-type responses (Mitarai 1964, 1965).

2.6 Stell Model of horizontal cell connectivity

Stell (1965) introduced the application of electron microscopy to the Golgi technique and used this technique to analyze the synaptic input to goldfish horizontal cells from photoreceptors. Stell (1967) demonstrated that external horizontal cells contact cones while the intermediate horizontal cells contact rods. Electron microscope studies of the carp retina (Witkovsky and Dowling 1969) confirmed the segregation of cone and rod input to external and intermediate horizontal cells in fish retina. Stell later showed that the inner horizontal cell described by Ramón y Cajal was not a separate type of horizontal cell, but the axon terminal of the external horizontal cells (Stell 1975). These axon terminals were the termination of the fine ($0.5 \mu\text{m}$) axons that Ramón y Cajal (1892) described but could never follow to their termination. Stell and Lightfoot (1975) identified three types of cone horizontal cells (classified H1, H2 and H3) and a single type of rod horizontal cell (designated RH).

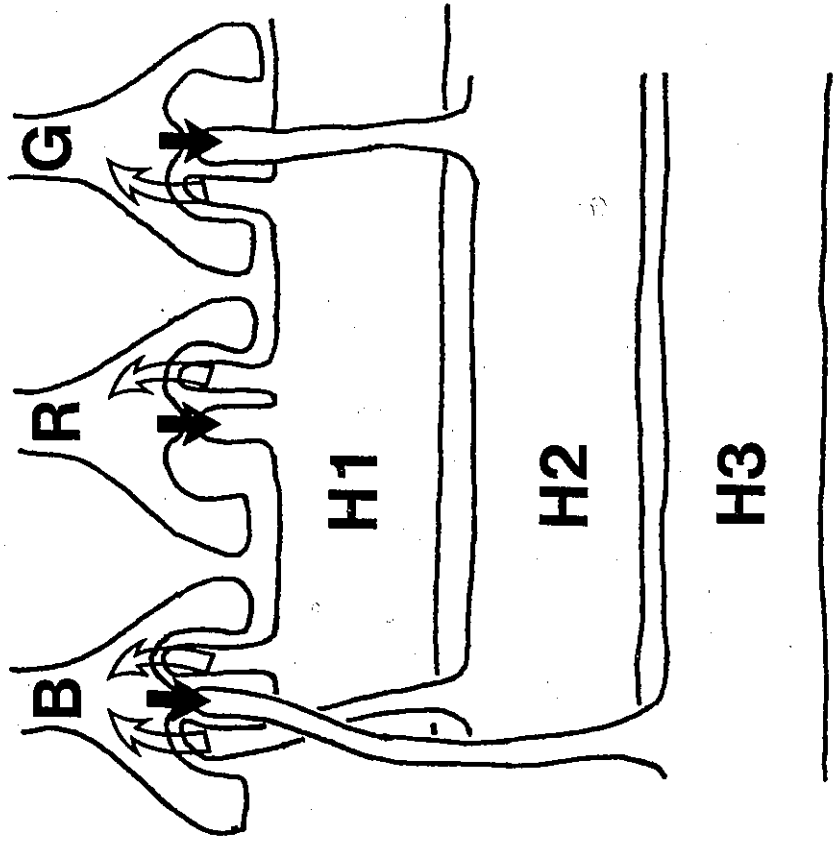
The knowledge of the goldfish cone mosaic (Engström 1960) and the correlation of cone morphology with photopigment type (Stell and Hárosi 1976) made it possible to identify the contacts made by cone type with specific Golgi-stained horizontal cells (Stell and Lightfoot 1975; Stell et al. 1975). A distinct pattern of horizontal cell contacts within the synaptic terminals of cones was found and is summarized in Fig. 1. By comparing this morphological pattern to the

chromatic responses of each of the three cone horizontal cells, it was proposed that the central dendritic elements of horizontal cells within the cone synaptic terminal were post-synaptic to cones whereas the lateral elements were pre-synaptic and sign-inverting to cones (Stell and Lightfoot 1975, Stell et al. 1975).

The lateral processes of horizontal cells provide an anatomical correlate for the depolarizing responses of the C-type horizontal cells through putative inhibitory contacts. H1 horizontal cells hyperpolarize to long wavelengths because of direct contacts with red cones. H2 horizontal cells depolarize to red stimuli because of a sign-inverting input from the lateral element of the H1 horizontal cell to the green cone which in turn contacts the central excitatory element of the H2 horizontal cell which provides a hyperpolarizing response to shorter wavelengths. H3 horizontal cells depolarize to green light because of the sign-inverting input of the H2 horizontal cell to the blue cone but the depolarizing response of the H2 horizontal cell to red is itself sign-inverted to yield hyperpolarizing responses in H3 horizontal cells to red light. Hyperpolarizing responses in H3 horizontal cells arise from direct contact with blue cones. Therefore, the response profile of H1, H2 and H3 horizontal cells to different wavelengths of light, from blue to red, are monophasic,

Figure 1 Diagram depicting the Stell Model of horizontal cell connectivity in the teleost retina.

The three types of cone horizontal cell (H1, H2, H3) receive excitatory input (solid arrows) from red (R), green (G) and blue (B) cones, respectively, at dendrites located centrally within the cone synaptic terminal. Each horizontal cell provides feedback to cones (open arrows) at dendrites positioned laterally within the cone synaptic terminal. H1 horizontal cells hyperpolarize at all wavelengths of light because the only feedback to the red cone is from the H1 horizontal cell. Feedback from the H1 horizontal cell depolarizes the green cone resulting in depolarizing responses of H2 horizontal cells to long wavelengths (red) light. The feedback from the H2 horizontal cell to the blue cone produces the depolarizing responses of H3 horizontal cells to green light.



①

biphasic and triphasic due to their unique synaptic connections to the three cone types.

All cone types are innervated by a H1 horizontal cell lateral element. The function of this feedback may be to adjust the operating range of the cones in response to changing levels of illumination (Baylor, Fuortes and O'Bryan 1971; Piccolino, Neyton and Gerschenfeld 1981). In this respect the H1 horizontal cell acts as a luminosity detector, hence the name L-type.

The organization of the rod synaptic terminal revealed only a single type of arrangement with rod horizontal cell processes placed laterally within the terminal (Stell and Lightfoot 1975; Stell et al. 1975).

In the cone synaptic terminal both the central and lateral horizontal cell elements have morphological specializations suggestive of post-synaptic sites (Stell and Lightfoot 1975). However, neither the central nor lateral elements appear to have any pre-synaptic specializations. It remains to be shown how the lateral horizontal cell elements could affect cones as suggested by the Stell Model. Recent evidence suggests that at least part of the inhibitory feedback is mediated by GABA (Section 2.18), released via a Ca^{2+} -independent, Na^{+} -dependent mechanism without submicroscopic specializations.

The Stell Model has been subject to criticism by Spekrijse and Norton (1970) who found that all three types of

cone horizontal cell had identical response dynamics. The Stell Model would predict that the dynamics should differ between horizontal cell types because of the multiple connections and differing pathways. It has also been suggested there is green cone input to the goldfish H1 horizontal cell (Yang, Tauchi and Kaneko 1983). The possibility that this is a reflection of electrical coupling between red and green cones has been rejected on the grounds that green stimuli affect red cone mediated H1 horizontal cell responses but not vice versa as would be predicted from cone coupling (Yang, Tauchi and Kaneko 1983). There is also pharmacological evidence that suggests a blue cone input to the H1 horizontal cell (Mangel, Ariel and Dowling 1985). Other electrophysiological studies in the carp have suggested there is input to and feedback from all types of cones and horizontal cells respectively (Kamermans, van Dijk and Spekreijse 1991). This conclusion is also consistent with the data showing that horizontal cell response dynamics are identical (Spekreijse and Norton 1970). It has been recently shown (Mangel et al. 1993) in dark-adapted white perch retina that a rod-like input to cone horizontal cells can be detected.

In defense of the Stell Model it can be noted that Spekreijse and Norton (1970) showed the latency of responses of carp retinal horizontal cell to red stimuli increased ($H3 > H2 > H1$) as predicted by the Stell Model. The fact that the

dynamics do not differ may suggest there is something special about the mechanism governing communication between the lateral elements in the cone synaptic terminal. The unexplained green cone input to the H1 horizontal cell (Yang, Tauchi and Kaneko 1983) might be due to rectified green to red cone coupling at the double-cone (Marchiafava, 1985). The functional relationship between the two members of the red-green double-cone, of which the red cone member is a major source of input to H1 horizontal cells (Stell 1967), is not completely understood (Baldrige et al. 1987). One requirement of the Kamermans Model (Kamermans, van Dijk and Spekrijse 1991) is there are different strengths of feedforward and feedback synapses. There is no known morphological correlate to account for this difference in synaptic strengths. The connectivity described by Stell and Lightfoot (1975) suggest that the response latencies for each type should be different. For example, the response latency to red light should increase in H2 horizontal cells compared to H1 horizontal cells because of the additional feedback synapse. Horizontal cells have different response latencies (Spekrijse and Norton 1970) consistent with the Stell Model but inconsistent with the Kamermans Model (Kamermans, van Dijk and Spekrijse 1991). Finally, the suggestion of rod input to cone horizontal cells (Mangel et al. 1993) does not necessarily imply a direct synaptic rod input to cone horizontal cells and may instead indicate coupling between rod

and cone photoreceptors after prolonged darkness. It is not possible to reconcile the pharmacological evidence suggesting a blue cone input to H1 horizontal cells (Mangel, Ariel and Dowling 1985) with the Stell Model.

2.7 Horizontal cell axons and axon terminals

Many studies have shown that the response properties of horizontal cell axon terminals are identical to the response properties of the horizontal cell somata to which they are connected (Kaneko 1970; Mitarai, Asano and Miyake 1974; Weiler and Zettler 1979). The fact that there is no decrement of response amplitude from somata to axon terminal suggests that the cable properties of the axon are unique. Whole cell patch clamp analysis of enzymatically dissociated goldfish horizontal cell axons revealed a decrease in the density of ionic channels of axon membrane compared to the membrane from the somata (Yagi and Kaneko 1988). This results in an extremely high membrane resistance of the axon making it plausible that conductance from the somata to the terminal (100 μm , Kouyama and Watanabe 1986) is passive (Yagi and Kaneko 1988). However, such a high membrane resistance would result in a significantly greater time constant resulting in an alteration in the dynamic properties of the response, an observation inconsistent with the passive conductance model of the horizontal cell axon (Yagi 1989). This raises the possibility that some unknown mechanism facilitates high-frequency signal conduction down the horizontal cell axon.

The function of the horizontal cell axon terminal is unknown. Unlike most species, the axon terminal of teleosts extends from the outer to inner retina. Anatomical studies in goldfish have demonstrated contacts with bipolar cells and other neurons in the inner nuclear layer (Marshak and Dowling 1987) and, in catfish, synaptic contacts with amacrine cell somata have been identified (Sakai and Naka 1985; 1986). This connection may represent an alternate pathway from the outer to inner retina to that provided by bipolar cells.

2.8 Horizontal cell receptive-field size

In addition to the varied chromatic response patterns of horizontal cells, early studies demonstrated that the receptive-field size of horizontal cell potential in carp was quite large (Tomita et al. 1958, Tomita 1965). In the tench retina it was shown that the horizontal cell response amplitude to a centred spot of light decays exponentially ($V=V_0e^{-kr}$) as it is moved laterally (Naka and Rushton 1967). A centred stimulus that resulted in a 10 mV hyperpolarizing response (V_0) only dropped to 2 mV (V) over a distance (r) of 400 μ m. Subsequently, Marmarelis and Naka (1972) reported much larger receptive fields of catfish horizontal cells, with a five-fold decrease in response as much as 2.5 mm distant from the receptive-field centre. They also suggested that the exponential decay function described by Naka and Rushton (1967) underestimated horizontal cell receptive-field size. Similar large receptive fields have been measured in the carp

retina (Teranishi, Negishi and Kato 1983). A comparison of cone and rod horizontal cell receptive-field size suggested that the rod horizontal cell receptive-field size was smaller (Kaneko 1970).

At about the same time, electron microscope studies revealed "specialized junctional complexes" (Yamada and Ishikawa 1965; Stell 1967; Witkovsky and Dowling 1969) between adjacent membranes of both horizontal cell somata and between horizontal cell axon terminals. Yamada and Ishikawa (1965) recognized these specializations as the morphological correlate of electrotonic communication observed by others (Robertson 1953; Furshpan and Potter 1959; Hama 1961; Bennett et al. 1963). The structures were termed "gap junctions" by Revel and Karnovsky (1967) to distinguish them from "tight junctions" which can have a similar appearance in conventional electron microscopy.

In freeze-fracture microscopy gap junctions have a distinct appearance of aggregations of transmembrane particles (Chalcroft and Bullivant 1970; Goodenough and Revel 1970; McNutt and Weinstein 1970). Gap junctions were first identified on horizontal cells by freeze-fracture in the all-rod retina of the skate (Fain, Gold and Dowling 1976) and subsequently between horizontal cell somata in the pikeperch (Witkovsky, Burkhardt and Nagy 1979), and horizontal cell axon terminals in turtle (Witkovsky, Owen and Woodworth 1983) and the goldfish (Wolburg and Kurz-Isler 1985; Baldrige, Ball and

Miller 1987). Since rod horizontal cells (H4) have never been specifically identified by freeze-fracture, it is not known if these cells have gap junctions though other evidence suggest they exist.

Horizontal cell dye-coupling was first demonstrated using Lucifer yellow in the turtle retina (Stewart 1978) and later between carp cone horizontal cells, rod horizontal cells and horizontal cell axon terminals (Kaneko and Stuart 1980, 1984). Dye coupling and conventional electron microscope studies showed that the coupling of horizontal cell somata and axon terminals was type specific. That is, H1 horizontal cells are coupled exclusively to other H1 horizontal cells, H2 horizontal cells coupled to H2 horizontal cells, and H3 horizontal cell coupled only to other H3 horizontal cells (Witkovsky and Dowling 1969; Kaneko and Stuart 1980, 1984). Kouyama and Watanabe (1986), however, demonstrated there are also gap junction contacts between carp horizontal cell axons and axon terminals of the same or different types although there are no physiological studies supporting the existence of such contacts.

Several different types of gap junction proteins have been identified (for review see Bennett et al. 1991), but there are no published reports demonstrating which type(s) of gap junction protein are found in retina. A single report from a meeting presentation showed immunoreactivity to connexin-43, the heart gap junction protein, in photoreceptors

and pigment epithelium in the goldfish retina (Baldrige, Mearow and Ball 1991) but not horizontal cells.

By establishing that horizontal cells are independent cellular elements (Ramón y Cajal 1892; Stell 1967) connected by gap junctions (Yamada and Ishikawa 1965; Witkovsky, Burkhardt and Nagy 1979) explains why the receptive-field size of teleost horizontal cells (Naka and Rushton 1967; Naka and Marmareis 1972) is approximately 10 times larger than their dendritic field size.

Current evidence suggests that the large receptive-field size of horizontal cells is due entirely to gap junction coupling since conventional chemical synaptic contacts from one horizontal cell to another have not been found (Yamada and Ishikawa 1965; Stell 1967; Witkovsky and Dowling 1969). Also, the large receptive-field size of horizontal cells is not due to coupling at the level of the photoreceptors. Although teleost photoreceptors make both electrotonic and chemical synaptic contacts with each other (Witkovsky, Shakib and Ripps 1974; Stell and Lightfoot 1975; Nishimura et al. 1982), teleost cones do not show the extensive spatial summation of horizontal cells (Tomita 1965). In turtle cones, Baylor, Fuortes and O'Bryan (1971) showed that hyperpolarizing one cone by current injection can hyperpolarize neighbouring cones up to 40 μm away. Because the typical diameter of a turtle cone is about 5 μm , this suggests coupling of up to 8

photoreceptors as a single electrical unit. However, this is much less than the receptive-field size of horizontal cells.

2.9 Chemical coupling of horizontal cells

Although chemical synaptic contacts connecting horizontal cells have not been identified, some recent evidence has suggested that horizontal cells may be chemically coupled by a GABAergic autofeedback system (Stockton and Slaughter 1991; Kamermans and Werblin 1992). GABA, released from a horizontal cell (see Section 2.18), is suggested to affect a chloride conductance gated by a GABA_A-receptor and depolarize the same horizontal cell or adjacent horizontal cells. The relative contribution of GABAergic chemical coupling to horizontal cell receptive-field size has not yet been determined.

2.10 Role of horizontal cells in construction of the receptive fields of bipolar and ganglion cells

2.11 Antagonistic centre-surround receptive fields of ganglion cells

Ganglion cells process the input from the retina and encode this information in the form of action potentials which are then transmitted to the brain along the axons of the ganglion cells that constitute the optic nerve. Hartline (1938) was the first to record extracellular responses from ganglion cells (in the frog) and identified three types based on the increase or decrease of ganglion cell action potential firing: ON-cells, OFF-cells and ON-OFF cells. In 1953 Barlow

found that the receptive fields of frog ON- and OFF-cells consisted of two roughly concentric circular regions. The central region responded to light as described by Hartline (1938). Outside this central region, however, the cell responded to light in the opposite way to the centre. A similar organization was found by Kuffler (1953) in cat and in goldfish ganglion cells by Wagner, MacNichol and Wolbarsht (1960) and Daw (1968).

Studies of goldfish retina have revealed many types of colour-coded ganglion cell receptive fields in addition to non-colour coded units as found in the frog and cat (Wagner, MacNichol and Wolbarsht 1960; Daw 1968; Beauchamp and Daw 1972; Spekrijse, Wagner and Wolbarsht 1972). The size of the receptive fields of goldfish ganglion cells are larger than the cat with a typical centre of about 1.5 mm (10° - 20° visual field) and the surround extending out to 5.5 mm (40° - 60° visual field) (Daw 1968).

Intracellular recordings from ganglion cells of mudpuppy, turtle, catfish and carp retina, revealed that membrane depolarization accompanies increased firing rates and membrane hyperpolarization accompanied decreased firing rates (Werblin and Dowling 1969; Naka and Ohtsuka 1975; Naka, Marmarelis and Chan 1975; Murakami and Shimoda 1977; Marchiafava and Weiler 1980).

2.12 Bipolar cells

Bipolar cells have somata located in the inner nuclear layer and send processes to the outer and inner plexiform layers (Ramón y Cajal 1892). Bipolar cells in teleost retinas were classified as giant and small by Ramón y Cajal (1892) who also observed that the giant bipolar cell was connected "particularly" with rods and the small bipolar cell "communicates" with cones. Stell (1967) confirmed Ramón y Cajal's observations about small bipolar cells, which synapse only with cones, but found that giant bipolar synapse with a few cones in addition to many rods. Bipolar cells extend dendrites into invaginations of the photoreceptor receptor terminal and have axon terminals that end in the inner plexiform layer.

Bipolar cells, like ganglion cells, have a centre-surround receptive field organization as first shown by Werblin and Dowling (1969) in mudpuppy and subsequently by Kaneko (1970) in goldfish. Bipolar cells, like photoreceptors and horizontal cells, respond to light with graded potentials. ON bipolar cells depolarize to light in the centre and hyperpolarize to light in the surround. OFF-bipolar cells hyperpolarize to light in the centre and depolarize to light in the surround.

The size of the receptive-field centre is about 100 μm in mudpuppy (Werblin and Dowling 1969) and 100-200 μm in the goldfish (Kaneko 1973). Bipolar cell receptive-field

surrounds are much larger, measuring between 1 and 2 mm. Like ganglion cells, bipolar cells in the goldfish and carp retina are colour-coded (Kaneko 1973; Kaneko and Tachibana 1983).

2.13 Construction of the bipolar cell receptive field

Werblin and Dowling (1969) and Werblin (1970) first suggested that the receptive-field centre of bipolar cells is due to the input they receive from photoreceptors and the receptive-field surround is due to input from horizontal cells. Werblin (1970) suggested that the strength of the mudpuppy bipolar cell surround, like the the horizontal cell receptive-field in fish (Naka and Rushton 1967), decreased exponentially with distance away from the receptive-field centre. The receptive-field size of fish horizontal cells was subsequently found to be much larger, up to several mm, than originally suggested by Naka and Rushton (1967) (Naka and Marmarelis 1972; Teranishi, Negishi and Kato 1983) and subsequent studies of bipolar cells (Kaneko 1973; Saito and Kujiraoka 1982) also reported larger (≥ 1 mm) receptive-field surrounds than previously suggested (Werblin 1970). Therefore, the large receptive-field size of horizontal cells is consistent with the proposed role of horizontal cells as providing the surround input to bipolar cells. However, the size of the bipolar cell receptive-field centre (300 μm in mudpuppy) does not match the size of the bipolar cell dendritic field (80-100 μm) (Dowling and Werblin 1969). Similar discrepancies were found in goldfish (Kaneko 1973).

One explanation is that bipolar cells are coupled, but by much less than are horizontal cells (Kujiraoka and Saito 1986), or that the size of the bipolar cell receptive-field centre reflects the extent of photoreceptor coupling.

The strongest evidence supporting the view that horizontal cells provide the surround input to bipolar cells comes from experiments where current was injected into horizontal cells and the response of bipolar cells examined. Studies in carp retina (Toyoda and Tonosaki 1978; Toyoda and Kujiraoka 1982) showed that hyperpolarization of horizontal cells produces surround-like responses in bipolar cells. Similar findings in catfish (Sakuranga and Naka 1985) and turtle (Marchiafava 1978) retina suggest that this is a general phenomenon of lower vertebrate retinas.

The anatomical details of how horizontal cells provide surround input to bipolar cells is still not completely understood. From the work of Stell (1967) it is apparent that horizontal cells could interact with cones (feedback), bipolar cell dendrites (feedforward), or both, within the cone terminal. Feedback has been demonstrated in the turtle (Baylor, Fuortes and O'Bryan 1971) and subsequently in the perch (Burkhardt 1977) and carp (Murakami et al. 1982a; 1982b). In these studies a cone was desensitized by a small spot of light and feedback observed as a depolarization of the cone by subsequent application of an annulus of light. Yang and Wu (1991) have reported that in addition to feedback,

there is horizontal cell feedforward input to bipolar cells that contributes about 1/3 of the surround response. Yang and Wu (1991) observed the feedforward component by blocking the cone inputs to the ON bipolar cell with L-2-amino-4-phosphonobutyrate (APB). Therefore, the cone→bipolar cell and horizontal cell→cone→bipolar cell pathways was blocked, leaving the cone→horizontal cell→bipolar cell pathway intact. After application of APB the ON bipolar responses to light (usually depolarizing) became hyperpolarizing, revealing the feedforward component.

2.14 Bipolar cell input to ganglion cells

Ramón y Cajal (1892) suggested that at least two cell types contact ganglion cells: bipolar cells and amacrine cells. In 1969 Werblin and Dowling suggested that sustained (ON or OFF) ganglion cells receive input primarily from bipolar cells whereas transient (ON-OFF) ganglion cells receive input from amacrine cells. This conclusion was reached by comparing the stimuli intensity vs. response properties of bipolar cells, amacrine cells and ganglion cells. The intensity vs. response curves of retinal neurons can be fitted by the Michaelis-Menten equation (Naka and Rushton 1967; Thibos and Werblin 1978):

$$R = \frac{R_{\max} I^n}{I^n + \sigma^n}$$

where R is response at intensity I, R_{\max} is the maximum response at saturation, σ is the intensity to give a half-

maximal response and m is the slope of the function. In mudpuppy, the slope determined from sustained ganglion cells ($m=1.4$) was very similar to the slopes determined from bipolar cells ($m=1.2-1.4$). Transient ganglion cells, however, had slopes ($m=3.4$) closer to values for amacrine cells.¹ The similarity of the Michaelis-Menten equation slopes of sustained ganglion cells and bipolar cells has been used as an argument for the dominant input of sustained ganglion cells being bipolar cells and not amacrine cells. This view is supported by experiments in the catfish retina where direct current injection into bipolar cells suggests a direct sign conserving signal transmission to ON and OFF sustained ganglion cells (Naka 1977).

Studies in mudpuppy retina suggest that the receptive fields of sustained ON and OFF ganglion cells are not determined entirely by bipolar cells of the same response type but are subject to "push-pull" antagonism (Belgum, Dvorak and McReynolds 1987). In this model ganglion cells receive input from bipolar cells with the same response type (ON or OFF) and also receive an inhibitory input from bipolar cells of the opposite response type carried by sustained amacrine cells.

¹ These values indicate that amacrine cells and transient ganglion cells show a change of 5% to 95% of maximum response with only 0.8 log units of light intensity. To change the responsiveness of bipolar cells and sustained ganglion cells a similar amount, a change of 1.5 log units or more intensity is required.

It is not known if a similar "push-pull" antagonism exists in the fish retina.

Since the receptive-field surround of bipolar cells is generated by horizontal cells, and bipolar cells provide either direct or amacrine cell-mediated input to ganglion cells, horizontal cells contribute to the receptive-field surround of ganglion cells. This is supported by experiments where current injected into horizontal cells produced surround-like responses not only in bipolar cells but also in ganglion cells of catfish (Naka and Nye 1971; Sakuranga and Naka 1985) and carp (van Dijk 1985). Extrinsic current injection into the rod horizontal cells of the all-rod retina of the cat shark also produced surround-like responses in ganglion cells (Naka and Witkovsky 1972).

2.15 Neurotransmitters and retinal cell physiology

2.16 Release of photoreceptor transmitter

Because photoreceptors (Tomita 1965) and horizontal cells (Svaetichin 1953) hyperpolarize to light, it was suggested (Trifonov 1968) that the photoreceptor transmitter was released during darkness when the cells were depolarized. This was consistent with known calcium-dependent neurotransmitter release from other neurons (Del Castillo and Katz 1954; Katz and Miledi 1967).

Trifonov and Byzov (1965; Byzov and Trifonov 1968) demonstrated in the turtle and carp retina that current that depolarized the cone terminal also depolarized L-type

horizontal cells. Therefore, cone transmitter is released onto horizontal cells during darkness keeping the horizontal cell membrane potential depolarized. Further evidence for dark release of photoreceptor transmitter comes from studies where synaptic transmission was blocked by substituting Mg^{2+} or Co^{2+} for Ca^{2+} (Dowling and Ripps 1973; Kaneko and Shimizaki 1975). Such treatments hyperpolarize the horizontal cell membrane to the K^+ equilibrium potential (E_K). Under these conditions the horizontal cell membrane potential is dependent on external K^+ alone (Kaneko and Shimazaki 1975). Therefore, the membrane potential of the horizontal cell in darkness is not the resting membrane potential of the cell but is depolarized by photoreceptor neurotransmitter released constantly in darkness.

In mudpuppy and dogfish horizontal cell membrane resistance increases during light stimulation (Toyoda, Nosaki and Tomita 1969; Kaneko 1971). Although there is one report of a similar finding in carp (Watanabe 1978) current injection studies are usually difficult to perform because of the low input resistance of horizontal cells that is a consequence of extensive gap junction coupling. It may also be difficult to detect a change in membrane resistance because of a voltage-dependent decrease in non-synaptic membrane resistance described by Trifonov, Byzov and Chailahian (1974) in carp horizontal cells. Nonetheless, the increase in membrane resistance in light suggests that the photoreceptor

transmitter increases one or more horizontal cell ionic conductances when released in the dark.

Studies of photoreceptor input to bipolar cells also support other studies demonstrating an increased release of photoreceptor transmitter in the dark. OFF-bipolar cells hyperpolarize to light with an increase in membrane resistance (Toyoda 1973). This suggests that OFF-bipolar cells, like horizontal cells, hyperpolarize to light because a photoreceptor transmitter-gated conductance decreases as the level of neurotransmitter release drops (Saito and Kaneko 1983). Like horizontal cells, OFF-bipolar cell membrane potential in the dark is kept depolarized by the constant release of photoreceptor transmitter in the dark. ON-bipolar cells, however, show decreased membrane resistance during a flash of light (Saito, Kondo and Toyoda 1979). As will be discussed later, this also is consistent with photoreceptor transmitter release in the dark.

2.17 Photoreceptor transmitter

In the early 1970's it was shown that the amino acids L-aspartate and L-glutamate depolarize carp and turtle horizontal cells and abolish the response of horizontal cells to light (Cervetto and MacNichol 1972; Murakami, Ohtsu and Ohtsuka 1972). Several lines of evidence suggest that glutamate is the true photoreceptor transmitter. First, the specific glutamate analogues ~~kainate and quisqualate~~ depolarize horizontal cells of dogfish (Sheills, Falk and

Naghshineh 1981), carp (Ariel et al. 1984), mudpuppy (Slaughter and Miller 1983a) and rabbit (Bloomfield and Dowling 1985) whereas agonists of aspartate (N-methyl-D-aspartate, NMDA) do not. (Because NMDA also acts at NMDA-type glutamate receptors, this result also suggests the absence of NMDA receptors on horizontal cells in these species). Second, isolated horizontal cells respond to low ($< 10 \mu\text{M}$) concentrations of glutamate and usually show no response to aspartate at the same low concentrations (Lasater and Dowling 1982; Ariel et al. 1984; Ishida et al. 1984). Anatomical studies also support the view that glutamate is the transmitter of at least some photoreceptors. In goldfish, rods accumulate glutamate and cones accumulate both glutamate and aspartate (Marc and Lam 1981) and many types of cones are glutamate immunoreactive (Ehinger 1989; Marc et al. 1990).

It has been suggested that the blue cone uses a transmitter other than glutamate. Mangel, Ariel and Dowling (1985) showed that the glutamate receptor antagonist D1- α -aminoadipate had a greater effect on red than on blue stimuli responses in carp H1 horizontal cells. By studying the change in horizontal cell membrane resistance, in retinas where horizontal cells were uncoupled by dopamine, Yasui and Yamada (1988) reported that blue stimuli did not increase resistance as much as red stimuli. Indeed, they found that blue stimuli sometimes decreased membrane resistance leading them to suggest that the blue cone contains a transmitter that

decreases horizontal cell membrane conductance. Anatomically, the blue cone only weakly accumulates glutamate (Marc and Lam 1981) and is weakly labelled by glutamate immunocytochemistry (Marc et al. 1990). It is, therefore, possible that there are photoreceptor transmitters other than glutamate.

The studies of the effect of glutamate on horizontal cells suggest that the glutamate receptors on most fish horizontal cells are of the non-NMDA type (Lasater and Dowling 1982; Rowe and Ruddock 1982; Ariel et al. 1984; Ishida et al. 1984). O'Dell and Christensen (1989) demonstrated the presence of NMDA receptors on catfish horizontal cells but it is not clear why catfish differ from other fish in this respect.

Patch-clamp studies of specific conductances of isolated fish horizontal cells show that glutamate increases an inward current with a reversal potential near 0 mV (Tachibana 1985). This conductance is due to a glutamate-gated channel of low selectivity permitting the movement of Na^+ , K^+ and to some extent Tris^+ , choline^+ , Ca^{2+} and Mg^{2+} (Tachibana 1985). The reversal potential near 0 mV is consistent with the depolarization of the horizontal cell membrane by glutamate release from photoreceptors in the dark.

In 1975 Murakami, Ohtuska and Shimazaki showed that glutamate hyperpolarized ON-bipolar cells and depolarized OFF-bipolar cells in carp retina. These results suggested that a single photoreceptor transmitter could account for the

responses of both types of bipolar cells. Subsequently, subtypes of glutamate receptors that govern the conduction increase of OFF bipolar cells and horizontal cells could be distinguished pharmacologically from receptors that govern the conduction decrease of ON-bipolar cells in mudpuppy (Slaughter and Miller 1981; 1983b). Only the ON-bipolar cell light response was blocked by the agonist 2-amino-phosphonbutyrate (APB) whereas OFF-bipolar cell and horizontal cell responses were blocked by the antagonist (\pm)cis-2,3 piperidinedicarboxylic acid (PDA). Eventually a pharmacological agent (O-phospho-D-serine) that could distinguish between the glutamate receptors of horizontal cells and OFF-bipolar cells was identified (Slaughter and Miller 1985).

OFF-bipolar cells are depolarized by glutamate by a similar mechanism as horizontal cells, an increase in a cation conductance with a reversal potential near 0 mV (Saito and Kaneko 1983; Attwell et al. 1987). ON-bipolar cells, however, probably respond to glutamate by a mechanism similar to the light-induced hyperpolarization of photoreceptors. Nawy and Jahr (1991) have shown in tiger salamander retina that binding of glutamate to the ON-bipolar cell glutamate receptor suppresses a cyclic GMP-activated current with a reversal potential near 0 mV (Shiells, Falk and Naghshineh 1981; Nawy and Copenhagen 1987; Atwell et al. 1987) by increasing the rate of cyclic nucleotide hydrolysis.

In goldfish and carp retinas ON-bipolar cell responses can be further characterized. In rod-dominated bipolar cells the depolarizing response to light is generated by an increase conductance to Na^+ (Saito, Kondo and Toyoda 1979; Nawy and Copenhagen 1987). In the red-cone dominated ON-bipolar cell the depolarizing response is generated by a conduction decrease to K^+ or Cl^- . Only the former is APB sensitive.

2.18 Horizontal cell transmitter

There is extensive evidence that fish H1 horizontal cells use GABA as a neurotransmitter. Goldfish H1 horizontal cells accumulate [^3H]-GABA (Lam and Steinman 1971) and [^3H]-muscimol (a GABA agonist) (Yazulla and Brecha 1980), and are immunoreactive for GABA (Mosinger, Yazulla and Studholme 1986). Goldfish H1 horizontal cells have been shown to make GABA from glutamate (Lam and Ayoub 1983) and the synthesizing enzyme for GABA, glutamic acid decarboxylase (GAD), has been localized immunohistochemically to H1 horizontal cells (Lam et al. 1979; Ball and Brandon 1986).

Several studies suggest that GABA is released from goldfish H1 horizontal cells by glutamate or darkness, when horizontal cells are depolarized (Schwartz 1982; Yazulla 1983; 1985). However, some of the release of GABA from toad and goldfish H1 horizontal cells does not appear to depend on the presence of external calcium (Schwartz 1982; Yazulla and Kleinschmidt 1983). Yazulla and Kleinschmidt (1983) proposed a Na^+ -dependent, carrier-mediated mechanism for GABA release

from H1 horizontal cells. This non-classical mechanism of neurotransmitter release may explain why no typical synaptic structures are observed between horizontal cell processes and the cone membrane in the cone terminal where horizontal cell to cone feedback or horizontal cell to bipolar cell feedforward are thought to occur.

GABA may be the transmitter that mediates feedback from H1 horizontal cells to cones. Consistent with this view are reports in turtle retina that GABA acts via GABA_A receptors to open Cl⁻ channel in certain cone types (Tachibana and Kaneko 1984). Since the reversal potential of GABA_A mediated Cl⁻ conductances is negative to the potential of cones in the dark, the effect of GABA would be to hyperpolarize cones tonically in darkness. In the presence of light, H1 horizontal cells are hyperpolarized and GABA release decreased. Consequently, Cl⁻ channels would close, acting to depolarize the cone in opposition to the hyperpolarization of the cone by light.

Because the feedback from horizontal cells to cones is also thought to produce the depolarizing responses of H2 and H3 horizontal cells, studies have suggested that GABA might mediate such feedback in fish retina. The GABA antagonist picrotoxin has been shown to block the depolarizing component of carp and roach C-type horizontal cell responses (Djamgoz and Ruddock 1979; Murakami et al. 1982b).

Although a role for GABA in mediating horizontal cell to cone feedback is persuasive, only the H1 horizontal cells have been shown to contain this transmitter. Interestingly, it has recently been reported that all three types of cone horizontal cell are GABAergic in the dragonet, a marine teleost (Van Haesendonck and Missotten 1992). It has also been demonstrated by Yazulla and Studholme (see Blazynski and Perez 1991) that a type of horizontal cell in the goldfish retina takes up [³H]-adenosine but is not GABA immunoreactive. This suggests that either the H2, H3 or rod horizontal cell might use adenosine as a neurotransmitter.

2.19 Bipolar cell to ganglion cell transmission

Bipolar cells contact amacrine cells and ganglion cells in the inner plexiform layer where the graded potentials of bipolar cells are passed on to ganglion cells. Famiglietti, Kaneko and Tachibana (1977) showed that the carp inner plexiform layer can be subdivided into a proximal ON region and a distal OFF region. Therefore, ON bipolar cells make synapses with ON ganglion cells in the proximal half of the inner plexiform layer and OFF bipolar cells make synapses with OFF ganglion cells in the distal half of the inner plexiform layer.

The transmitter of bipolar cells is likely to be glutamate. Although goldfish bipolar cells show poor uptake of [³H]-glutamate (Marc and Lam 1981), both ON- and OFF-bipolar cells are glutamate immunoreactive (Marc et al. 1990).

Using isolated goldfish horizontal cells as a bioassay for glutamate release from bipolar cells, Tachibana and Okada (1991) showed that depolarization of isolated goldfish ON-bipolar cells by current injection mimicked the effect of glutamate application onto horizontal cells.

Inhibitory amacrine cell input to sustained ganglion cells, carrying the signal from bipolar cells of the opposite response type (Belgum, Dvorak and McReynolds 1987), has not been demonstrated in the fish retina. However, in the mudpuppy such amacrine cell input may be mediated by GABA (Belgum, Dvorak and McReynolds 1984).

2.20 Summary of retinal circuitry

Light hyperpolarizes photoreceptors resulting in a reduction of photoreceptor glutamate release. As a consequence horizontal cells and OFF-bipolar cells hyperpolarize and ON-bipolar cells depolarize. Horizontal cells provide both feedback inhibition to cones and feedforward inhibition to bipolar cells, producing an antagonistic receptive-field surround. The responses of the surround are opposite to the responses of the bipolar cells produced by photoreceptor input. At least one type of horizontal cell uses GABA as the inhibitory neurotransmitter to produce the surround input. The size of the receptive-field surround is large as a consequence of the extensive gap junction coupling of horizontal cells. The receptive field properties of the bipolar cell are passed on to sustained

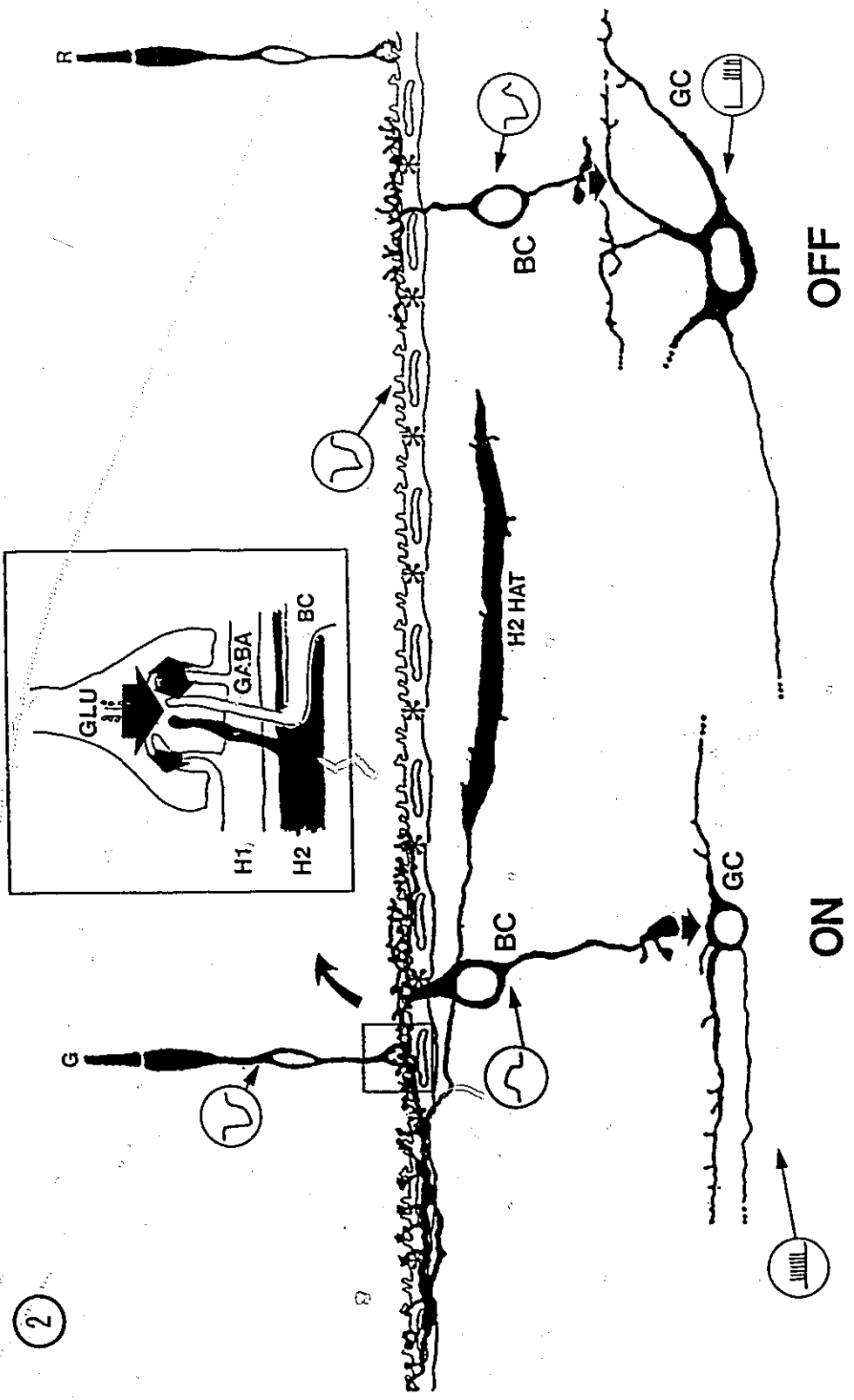
ganglion cells of the same type (ON or OFF). This basic plan of retinal circuitry is summarized in Figure 2.

2.21 Functional significance of antagonistic centre-surround receptive fields

Unlike the film of a camera, the retina does not simply encode the absolute level of luminosity from each part of the image falling on it. One reason this is so is due to the enormity of such a task. The visual system can function over some 10^{10} units of light intensity (Dowling 1987). If the signal from the retina was simply an absolute reflection of intensity, the ganglion cells would have to encode an equivalent number of states (and this does not include coding for chromatic information). Instead, antagonistic centre-surround receptive fields permit the ganglion cells to signal the brain about the difference in luminosity in a given region of the visual field compared to the average luminosity in a larger surrounding region. Therefore, the only information that needs to be encoded is where differences are detected.

Centre-surround receptive field organization can also enhance the quality of the visual image. This function is often discussed in association with the suggestion that the lens of the eye degrades the quality of the visual image due to chromatic and spherical aberration (Rodieck 1973). Therefore, an antagonistic centre-surround receptive field organization could sharpen the degraded image. However, in fish the quality of the lens is reported to be quite good

Figure 2 Circuitry of the teleost retina mediating form perception. The ON bipolar cell (BC) receives glutamatergic (GLU) input from the green (G) cone via dendrites positioned centrally within the cone synaptic terminal (inset, large arrow). Red (R) cones provide input (also glutamatergic) to the H1 horizontal cell (HC) network which is coupled extensively by gap junctions (*). GABAergic H1 HC dendrites, positioned laterally within the G cone synaptic terminal, produce the BC receptive-field surround by feedback and feedforward (small arrows). The output of ON BCs is to ON ganglion cells (GC) in the proximal inner plexiform layer and uses glutamate as a transmitter (arrows). An OFF-BC and OFF-GC, which synapse in the distal IPL, are also shown. Possible "push-pull" inhibitory input to ganglion cells from amacrine cells (Belgum, Dvorak and McReynolds 1987) is not shown. From the circuitry depicted in the diagram, the depolarizing responses of H2 HCs to red light can be explained. Red light hyperpolarizes the R cone, decreasing glutamate release and hyperpolarizing the H1 HC network. Dendrites of H1 HCs provide feedback that depolarize the G cone due to reduced GABA release (see inset). This results in increased glutamate release from the G cone thereby depolarizing the H2 horizontal cell. Responses of cells depicted by circular insets are to a spot of white light. H2 HAT = H2 horizontal cell axon terminal. Drawings of some cells by R.E. Marc and responses were taken from *Trends in Neuroscience*, volume 9.



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(Fernald 1990; Sivak 1990). In many species, the optical properties of the lens exceed the properties of the retina with respect to acuity. For example, the resolving power of the African cichlid lens is some ten times greater than the resolving power of the retina based on the spacing of the cones (Fernald and Wright 1985). There is, however, the potential for spatial acuity loss in the retina due to photoreceptor coupling and anatomical convergence of many cones on a single bipolar cell and bipolar cells to even fewer ganglion cells (Stell and Koch 1984). Measurements suggest that acuity in fish is maintained at about what would be expected from coupled cones (Stell and Koch 1984). The level of acuity established at the photoreceptors is maintained by the antagonistic centre-surround receptive field organization.

The neural representation of a point image on the retina, blurred by photoreceptor coupling and convergence, can be represented by a Gaussian function (Fig. 3A). At the level of bipolar cells this degraded image is sharpened by lateral inhibitory input from horizontal cells that can also be described by a Gaussian function (Fig. 3A). This "difference of two Gaussian functions" (Rodieck and Stone 1957) models one Gaussian, stronger, narrow and excitatory, as the receptive-field centre, and the other, weaker, wide and inhibitory, as the receptive-field surround. The inhibitory field sharpens the edges of excitatory centre (see Fig. 3B). Acuity is also degraded by the blurring of the image because as two single

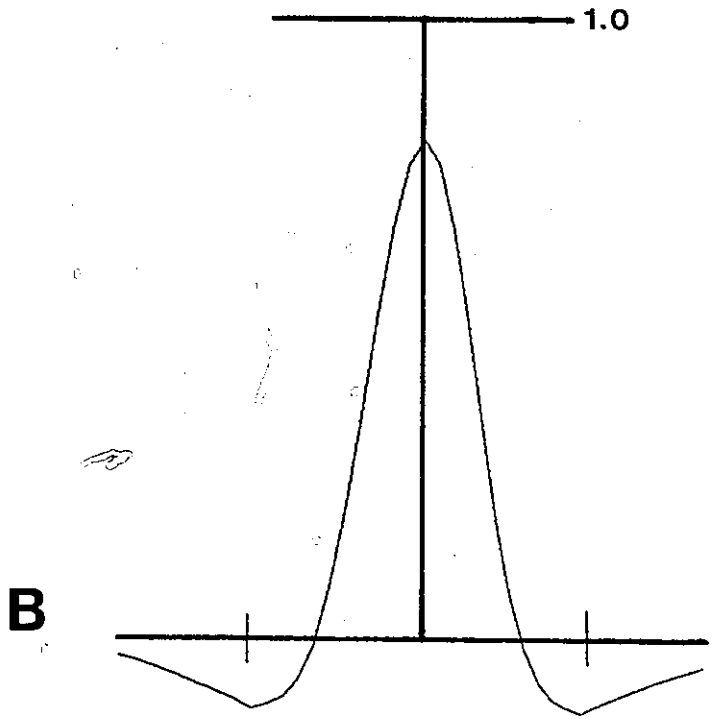
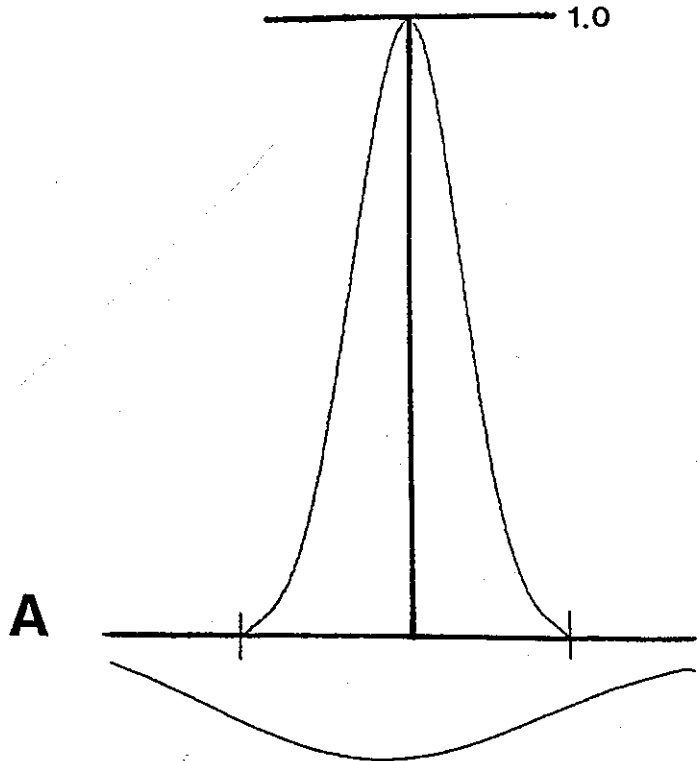
Gaussian distributions come close the blurred borders fuse becoming indistinct (Fig. 3C). In the difference of Gaussian model, the inhibitory fields combine to enhance the difference between two points. This increases resolution and, therefore, acuity (Fig. 3D).

Antagonistic centre-surround receptive fields are not unique to vertebrate retinal bipolar and ganglion cells. Such receptive fields have also been described from recording of neurons in post central gyrus of cerebral cortex, neurons associated with somatic sensation (Mountcastle 1957). In the same way that visual stimuli are sharpened in the retina, the sensation of touch and two-point discrimination can be refined by lateral inhibition. Lateral inhibition is a common feature of sensory systems. For example, in the olfactory bulb lateral inhibitory input from periglomerular cells modify the direct input of the olfactory receptors to the mitral cells in a way analogous to the modification of photoreceptor input to bipolar cells by horizontal cells (Shepherd 1978).

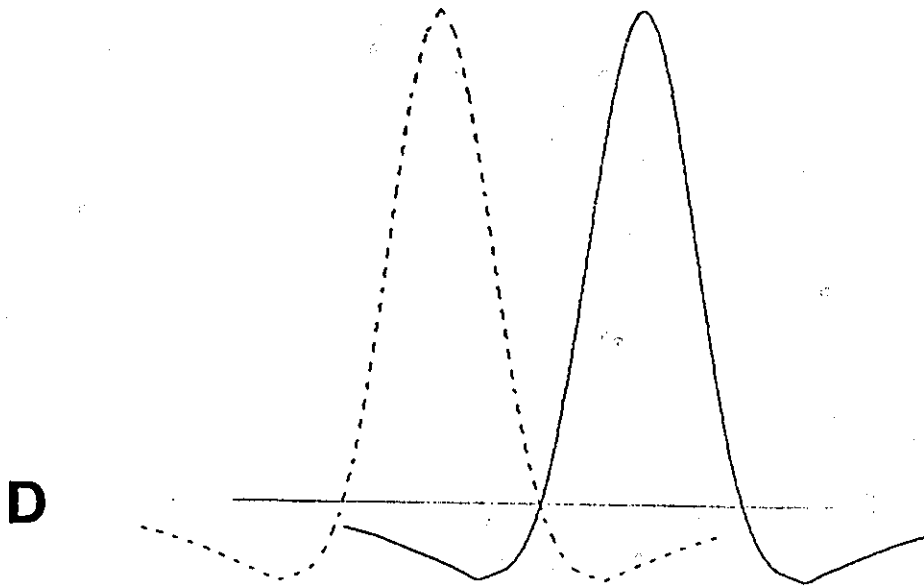
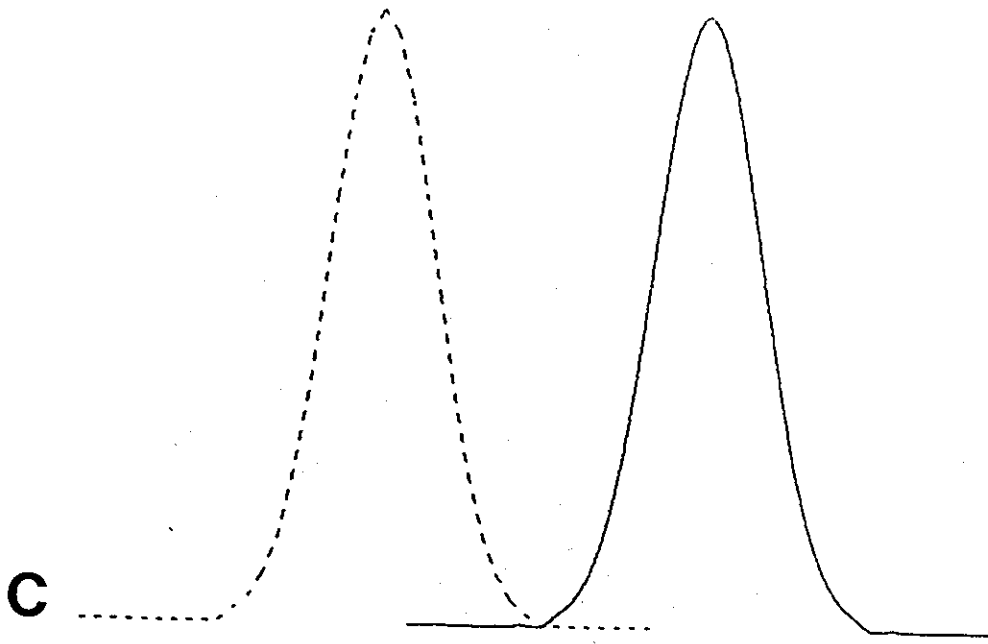
Antagonistic centre-surround receptive fields also endow cells with a mechanism for light-dark border detection. This was first realized by Hartline and Ratliff (1957) in the invertebrate (*Limulus*) visual system but has been subsequently generalized to include any cell receiving lateral inhibitory input. Consider three different cells, each with central excitatory and lateral inhibitory input, at three different locations: one in the light, one right at the light-dark

Figure 3 Difference of Gaussians model of ganglion cell receptive field organization. (A) The upper curve is a representation of a point image on the retina at the level of photoreceptor input to the bipolar cell. The lower curve represents the horizontal cell input. (B) This is the sum of the upper and lower curve in (A) and represents the receptive field of a bipolar or ganglion cell. In the centre is an excitatory field narrowed by the inhibitory field surrounding it. Note that the amplitude of the excitatory centre is necessarily reduced by inhibitory input. (C) If the ability to resolve two points is taken as the region where the signal level between the points is zero, the presence of an inhibitory surround decreases this distance (D). Therefore, resolution is increased by the presence of an inhibitory surround but at the expense of amplitude and sensitivity.

The excitatory curve was generated from Ae^{-ax^2} where $A=1$, $a=1$ and x was arbitrary distance units. The inhibitory curve was generated from Be^{-bx^2} where $B=0.2$ and $b=0.1$. The abscissa represents distance and the ordinate direction amplitude.



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border and one in the dark. The cell in the light does not respond maximally because it receives significant inhibitory input that reduces or even cancels the excitatory input. The cell in the dark does not respond either because it is not excited. However, the cell at the border responds the most because it is excited by the light but inhibited less because part of the inhibitory field is in the dark. This property has been used to explain the Mach band phenomenon (Ratliff 1965). If a series of strips of different intensity are placed in series from darkest to brightest from left to right, within each strip it will appear as if the intensity decreases from left to right even though the intensity is actually constant. This is because the inhibitory input from the right is stronger than from the left.

Exactly the same kind of processing that has been described for luminance alone also occurs with respect to colour. The colour of an object is not determined by identifying the wavelength characteristic for each point in visual space but by comparing the strength of a particular wavelength in one local part of the scene to a larger part of the scene.

In the difference of two Gaussians model of ganglion receptive fields, in the detection of borders and in colour processing, the retina compares one part of the visual world to the average properties of the visual world. Although these comparisons have important functional consequences for inner

retinal neurons, the components of these comparisons are generated in the outer retina by interactions between photoreceptors, bipolar cells and horizontal cells. The collection of the average properties of the visual world are a direct consequence of horizontal cells and, in particular, of horizontal cell coupling.

3.0 Adaptation

In the dark photoreceptors can respond to a 10^3 change in light intensity (Fain and Dowling 1973). However, the range of light intensity that animals that are active in day and night encounter may be as much as 10^{10} (Dowling 1987). To adjust to this range of intensities animals regulate the amount of light reaching the photoreceptors either by regulating the opening of the iris diaphragm or, as in fishes, by movement of screening pigments. These changes account for less than a 10 fold difference in intensity (Dowling 1987). An additional mechanism is for photoreceptors to have different sensitivities. Such a situation exists in Duplex retinas where one population of photoreceptors (rods) has a greater sensitivity to light than the other (cones) (Fain and Dowling 1973). It is possible, therefore, to account for approximately 10^6 units of dynamic range based on the basic properties of both the eye and photoreceptors. The remaining 10^4 units of dynamic range is accounted for by adapting the photoreceptors and neural network.

The retina can adjust both to decreasing light levels (dark-adaptation) and increasing light levels (light-adaptation). As discussed previously this is due, in part, to shifts between the cone and rod systems. However, the operating range of each photoreceptor type can also be altered.

3.1 Photoreceptor adaptation

The photoreceptor outer segment dark current is primarily due to the inward movement of Na^+ but the channel that permits the movement of Na^+ is also somewhat permeable to Ca^{2+} . During a flash of light, when the channels are closed, the influx of both Na^+ and Ca^{2+} are decreased. Yau and Nakatani (1984) showed that a Ca^{2+} - Na^+ exchanger actively pumps Ca^{2+} out of the cell. The activity of the pump that removes Ca^{2+} from the cell is not decreased by light. Therefore, during a flash of light there is a decrease in the internal concentration of Ca^{2+} in the photoreceptor due to both the reduction of Ca^{2+} influx and the continued operation of the Ca^{2+} pump (Yau and Nakatani 1985; McNaughton, Cervetto and Nunn 1986).

The concentration of intracellular Ca^{2+} may play a role in photoreceptor adaptation. It has been shown that Ca^{2+} inhibits guanylate cyclase and activates phosphodiesterase (Lolley and Racz 1982). Therefore, when $[\text{Ca}^{2+}]_i$ drops in the light, guanylate cyclase activity is increased and phosphodiesterase activity decreases resulting in an increase

in the level of cyclic GMP. These changes are opposite to the effect of light mediated by transducin that decreases cyclic GMP levels. As a result, the membrane potential of a photoreceptor in the light is adjusted (depolarized) back toward the dark level thereby increasing its operating range.

The effect of $[Ca^{2+}]_i$ on phototransduction can explain the physiological observation that photoreceptors can continue to respond to stimuli in the presence of background light that should be saturating. Because the dynamic range of photoreceptors in the dark is about 3 log units, a stimulus 3 log units brighter than the threshold response should saturate the photoreceptor so no additional response is detected even if the stimulus is brighter than 3 log units. Although photoreceptors hyperpolarize to a bright light (intensity $>10^3$), the amplitude of the hyperpolarization is not sustained. Within a few seconds to several minutes the photoreceptor depolarizes and becomes responsive once again (Dowling and Ripps 1970; Baylor and Hodgkin 1974; Kleinschmidt and Dowling 1975). It has been suggested that this recovery of photoresponse is due to the calcium-mediated increase in cyclic GMP that adjusts the membrane potential of the photoreceptor (Nakatani and Yau 1988).

In the presence of background light, the intensity vs. response functions of photoreceptors shift so the cells can operate at higher intensities (Dowling and Ripps 1970; Normann and Werblin 1974; Kleinschmidt and Dowling 1975; Fain 1976).

The dynamic range of the photoreceptor does not change but the cell operates over an increased minimum and maximum intensity.

The mechanism of these changes in photoreceptor sensitivity are different from the recovery of the photoreceptor membrane potential related to changing $[Ca^{2+}]_i$. The time course of the shift of the voltage-intensity relationship can be faster than the recovery of membrane potential suggesting that it is independent of membrane potential (Dowling and Ripps 1971, 1972). As a result it has been argued there is another internal messenger, other than Ca^{2+} , that regulates this form of adaptation (Bastian and Fain 1979; Lamb, McNaughton and Yau 1981). One explanation, proposed for rods, is that bleached rhodopsin reduces the sensitivity of unbleached rhodopsin (Dowling 1987; Leibovic 1990).

Feedback from luminosity-type horizontal cells, such as the H1 horizontal in the fish, can also regulate the operating range of cones (Baylor, Fuortes and O'Bryan 1971; Piccolino, Neyton and Gerschenfeld 1981). In the fish feedback is likely mediated by the transmitter GABA (Djamgoz and Ruddock 1979; Murakami et al. 1982a).

The adaptation of rods extends the range of photoreceptor responsiveness to about 10^5 levels of intensity. This is near the threshold of cone photoreceptors which, including their adaptive mechanism, accounts for the next 10^5 levels of intensity. Therefore, photoreceptor adaptation

allows the retina to operate over the full range of light intensities naturally encountered.

3.2 Changes in ganglion cell receptive field organization

In addition to photoreceptor adaptation, which permits detection of visual stimuli under a variety of light levels, there are also changes in how the retina processes this information that depend on the ambient level of illumination.

It was first noted in the cat that the size and strength of the surround component of sustained ganglion cell receptive fields decreases in the dark (Barlow, FitzHugh and Kuffler 1957). Subsequently, similar changes were observed in the goldfish (Raynauld, Laviolette and Wagner 1979) although others report the presence of rod input in the surround after dark-adaptation (Adams and Afandor 1971; Beauchamp and Daw 1972). Since horizontal cells provide the surround input to bipolar cells and, therefore, contribute to the receptive-field surround of ganglion cells, changes in the ganglion cell receptive-field surround may reflect changes of the activity of cone horizontal cells.

3.3 Changes in horizontal cell activity with adaptation

3.4 Theory: Changes of horizontal cell coupling and responsiveness

The large receptive-field size of horizontal cells is due to extensive gap junction coupling. It is not necessarily the case, however, that changes in horizontal cell receptive-field size are due exclusively to changes in gap junction

coupling. It is possible to model the decay of horizontal cell response from centre to periphery from the response at the centre and a characteristic constant (Naka and Rushton 1967). Lamb (1976) suggested that the receptive-field size of horizontal cells could be described by a "space constant" (λ) which, analogous to cable theory, was a function not only of gap junction coupling (R_1) but also of membrane resistance (R_m): $\lambda = \sqrt{R_m/R_1}$. Therefore, a change in horizontal cell receptive-field size could be due to either an increase in gap junction resistance (R_1), a decrease in membrane resistance, or both (Lamb 1976; Piccolino et al. 1982).

One way to determine if R_1 changes when the receptive-field size decreases is to determine if dye coupling is also reduced. However, an alteration in dye coupling does not rule out the possible involvement of R_m as part of the mechanism that results in the decrease of receptive-field size. Changes in the responses of horizontal cells to different stimuli types also reveal information about relative changes of gap junction and membrane resistance. The response to small spot or slit stimuli in the centre of the horizontal cell receptive field should increase when horizontal cell gap junction resistance increases because the effective input resistance increases (Lamb 1976). In effect, less current passes through gap junctions to neighbouring cells leaving more current to pass through the membrane near the impaled cell resulting in an increased voltage response. Responses to full-field

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stimuli should not change markedly when only gap junction resistance changes. This is because when all the photoreceptors are illuminated the horizontal cells should be equipotential. However, the response to full-field stimuli will decrease when membrane resistance decreases. A reduction in membrane resistance may not be detected by small spot stimuli since the area of membrane involved is too small. In summary, changes in R_i are revealed by changes in the response to small stimuli, changes in R_m are revealed by changes in full-field stimuli.

One method of assessing horizontal cell receptive-field size is to compare horizontal cell responses using annulus stimuli. Such stimuli can clearly demonstrate changes in horizontal cell receptive-field size but do not distinguish between changes in gap junction coupling and changes in membrane resistance. Reduced response to an annulus could be due to either reduced current flow between cells (gap junction change), increased current leakage out of cells (membrane resistance), or both.

The use of spot, annuli and full-field stimuli provide qualitative measures of changes in horizontal cell receptive-field size. Quantitative measures of changes in horizontal cell receptive-field size can be made by translating a small spot or slit of light across the retina and determining the space constant (Naka and Rushton 1967; Lamb 1976). However, this value alone does not indicate whether a change in

receptive-field size is due to a change in gap junction coupling or membrane resistance. However, Piccolino, Neyton and Gerschenfeld (1984) showed that the shape of the curves describing response decay with distance were different depending on whether R_m or R_i changed. Response to small stimuli near the cell being recorded should increase as R_i increases due to the increase in input resistance. With decreased R_m responses to small stimuli near the centre of the horizontal cell receptive field will either not change, because of the small area of membrane involved, or decrease slightly. In both cases, response to stimuli presented distant from the centre will decrease.

The changes in horizontal cell response to different stimuli when R_i , R_m , or both are changed are summarized in Table 2.

3.5 Changes in horizontal cell receptive-field size during adaptation

In the fish retina the responsiveness and receptive-field size of horizontal cells is decreased after prolonged darkness (Mangel and Dowling 1985; Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988a, 1988b). Dark-adapted retinas exposed to light show dramatic increases in responsiveness, a process called "light-sensitization" (Yang, Tornqvist and Dowling 1988a). These changes were assessed by comparing the responses of horizontal cells to increasing sizes of spot stimuli up to full illumination of the retina.

Table 2.--Changes in responses of horizontal cells to different stimuli under different conditions

	Small spot over centre	Full-field	Annulus or small spot distant from centre
Reduce gap junction coupling ($\uparrow R_1$)	increase	no change	decrease
Reduce membrane resistance ($\downarrow R_m$)	no change or decrease	decrease	decrease
both	increase	decrease	decrease

The responses of horizontal cells to small spot stimuli after dark-adaptation were greater than after light-sensitization (Tornqvist, Yang and Dowling 1988). This suggests that the gap junction coupling between horizontal cells is decreased in the dark. Conversely, the response to full-field illumination was decreased in the dark and dramatically increased after light-sensitization. This suggests that membrane resistance of horizontal cells is also decreased in the dark.

Similar changes in the rod horizontal cells of carp and the all rod retina from the cat shark have been reported by Villa, Bedmar and Barón (1991) but not in the rod horizontal cells of skate (*Raja*), another all-rod retina (Qian and Ripps 1992).

The changes in receptive-field characteristics of cone and possibly rod horizontal cells that occur after light adaptation are consistent with horizontal cells being the source of the surround input to sustained ganglion cells and the observed decrease of ganglion cell receptive-field size in the dark.

3.6 Dopaminergic interplexiform cells

A type of cell that corresponded to the "special stellate cells" of Ramón y Cajal (1892) was identified by Ehinger, Falck and Laties (1969) using the Falk-Hillarp method. Originally described as a subtype of amacrine cell, these cells were named interplexiform after Golgi studies

demonstrated that these cells have processes that pass into both plexiform layers (Gallego 1971). Virtually all vertebrate retinas have been found to contain interplexiform cells (Gallego 1971; Boycott et al. 1975; Oyster and Takahashi 1977; Frederick et al. 1982; Kleinschmidt and Yazulla 1984; Brunken, Witkovsky and Karten 1986; Oyster et al. 1986).

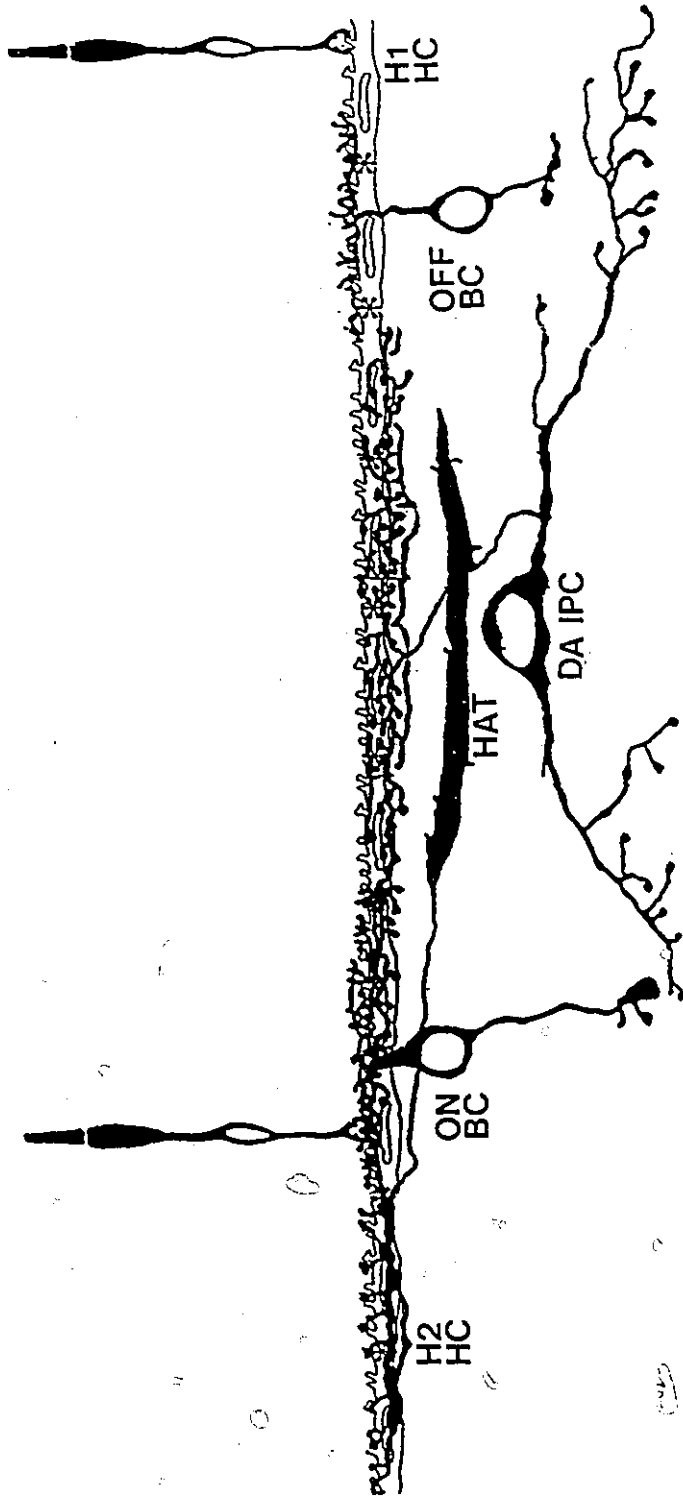
The Falk-Hillarp method reveals the presence of monoamines by a histochemical reaction. It was subsequently found that the dominant catecholamine in the fish retina is dopamine. Evidence that dopamine is contained within fish interplexiform cells comes from studies demonstrating that interplexiform cells are immunoreactive for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine from tyrosine (Yazulla and Zucker 1988) and have a high-affinity uptake system for [³H]-dopamine (Sarthy and Lam 1979; Marc 1982). Light and electron microscopy have revealed that dopaminergic interplexiform cells receive input mostly from amacrine cells in the inner retina and make extensive contacts with external (cone-type) horizontal cell somata and occasionally bipolar cells in the outer retina (Ehinger, Falck and Laties 1969; Dowling and Ehinger 1978; Yazulla and Zucker 1988). There are also occasional contacts of dopaminergic interplexiform cells onto presumed amacrine cell processes in the inner plexiform layer. No study of goldfish or carp dopaminergic interplexiform cells has found contacts onto photoreceptors, even at the synaptic terminal, but there is a

report that such contacts do occur in catfish and blue acara retinas (Wagner and Wulle 1990, 1992). The significance of these contacts are not known. An illustration of the general morphological characteristics of the dopaminergic interplexiform cell is given in Fig. 4.

3.7 Effects of dopamine on horizontal cell receptive-field size

The first physiological study of the effect of dopamine on fish retina showed that it depolarized cone type horizontal cells by 5-10 mV and reduced the responsiveness of the cells to light (Hedden and Dowling 1978). Dopamine did not have any noticeable effect on the rod type (H4) horizontal cells. Bipolar cells were also affected by dopamine. The membrane potential of ON-type cells were hyperpolarized, the depolarizing ON-response to a spot stimulus increased and the hyperpolarizing response to an annulus decreased (Hedden and Dowling 1978), indicating that the antagonistic surround of the bipolar cell was reduced. The surround responses of cones were also reduced by dopamine, but dopamine had no effect on the membrane potential of the cone (Hedden and Dowling 1978). The effects of dopamine on bipolar cells and cones, as described above, are consistent with the action of dopamine on horizontal cell activity rather than a direct action of dopamine on bipolar cells or cones. Since horizontal cells generate the receptive field surround of bipolar cells, the loss of the surround is consistent with an action of dopamine

Figure 4 Diagrammatic illustration of the dopaminergic interplexiform cell. The somata of the dopaminergic interplexiform cell is found in the inner nuclear layer. Processes project both to the inner plexiform layer and the outer plexiform layer. In the latter case, the processes form contacts with horizontal cell somata. Figures of cells based on drawings by R.E. Marc.



on horizontal cells. Similarly, the effect of dopamine on cones is most likely due to the action of dopamine on horizontal cell to cone feedback.

Negishi and Drujan (1979a, 1979b) ultimately showed that dopamine affected the receptive-field size of H1 horizontal cells in the carp retina. Dopamine increased the response of H1 horizontal cells to small spot stimuli and decreased the response to annulus stimuli. This led them to suggest that dopamine increased the resistance of horizontal cell gap junctions. This view was substantiated by studies demonstrating that H1 horizontal cell dye coupling was reduced after treatment with dopamine (Negishi, Teranishi and Kato 1983; Teranishi, Negishi and Kato 1983). Teranishi, Negishi and Kato (1984) subsequently showed that the receptive-field size and dye-coupling of all types of cone horizontal cells (H1, H2, H3) were reduced by dopamine. Mangel and Dowling (1985) demonstrated that dopamine increased the responses of horizontal cells to small spot stimuli and decreased the response to full-field stimuli in goldfish retina. This evidence suggested that both gap junction coupling and membrane resistance of horizontal cells are affected by dopamine. Horizontal cells are coupled at both their somata and axon terminals. The receptive-field size and dye-coupling of axon terminals of H1 horizontal cells have been reported to be unaffected by dopamine (Hida, Negishi and Naka, 1984; Teranishi, Negishi and Kato 1984).

Although dopaminergic interplexiform cells are thought to have no direct contact with rod horizontal cells and dopamine is reported to have no effect on rod horizontal cell membrane potential (Hedden and Dowling 1978), a recent study finds that dopamine depolarizes and reduces the receptive-field size of rod horizontal cells in the carp (Yamada et al. 1992). However, in the all-rod retina of the skate that dopamine has no affect on rod horizontal cells (Qian and Ripps 1992).

There is evidence that the manipulation of retinal dopamine levels *in vivo* also affects horizontal cell receptive-field size and dye-coupling. In retinas where the dopaminergic interplexiform cells are lesioned with 6-hydroxydopamine, the receptive-field size and dye-coupling is increased compared to control unlesioned retinas (Cohen and Dowling 1983; Teranishi, Negishi and Kato 1983, 1984).

3.8 Mechanism of action of dopamine on horizontal cell receptive-field size and responsiveness

The demonstration of a dopamine-sensitive adenylate cyclase in the brain (Kebabian, Petzgold and Greengard 1972; Iversen 1975), has been shown to be associated with the D1 dopamine receptor (Kebabian and Calbe 1979). Others have demonstrated dopamine-dependent increases in cyclic AMP in the mammalian retina (Brown and Makman 1973; Schorderet 1977). In the carp retina dopamine also increased cyclic AMP production, an effect that could be blocked by D1 dopamine receptor

antagonists (Watling and Dowling 1981; Dowling and Watling 1981). Dopamine was also shown to increase cyclic AMP in fractions of isolated horizontal cells (Van Buskirk and Dowling 1981). These results suggest that the effects of dopamine on fish horizontal cells are mediated by a D1 dopamine receptor.

In carp retina, Teranishi, Negishi and Kato (1983) showed that the membrane permeable analogue of cyclic AMP, dibutyryl cyclic AMP, mimicked the effect of dopamine on horizontal cells. Similar results were found in turtle retina (Piccolino, Neyton and Gerschenfeld 1984). This study also showed that a variety of D1 dopamine receptor antagonists blocked the effect of dopamine. The moderately selective (D2>D1) dopamine receptor antagonist haloperidol (Mangel and Dowling 1987) and the selective D1 dopamine receptor antagonist SCH-23390 (Yang, Tornqvist and Dowling 1988) have also been shown to block the effects of dopamine on fish horizontal cells.

Direct evidence of the effects of dopamine and cyclic AMP on horizontal cell coupling comes from work done on isolated fish horizontal cells. Lasater and Dowling (1985a, 1985b) found that coupled pairs of isolated horizontal cells were uncoupled by both dopamine and cyclic AMP. In these studies, electrical coupling could be measured directly by impaling both cells and measuring the extent of the current passed from one cell to another. An additional finding of

this work was that coupling between isolated pairs of horizontal cells did not exhibit voltage-dependence suggesting that changes in horizontal cell membrane potential should not affect horizontal cell gap junction coupling.

Dopamine can reduce horizontal cell receptive-field size by reducing both gap junction coupling and membrane resistance. In addition to dye coupling studies, definitive evidence that dopamine and cyclic AMP reduce gap junction coupling came from patch-clamp studies in which the properties of single gap junction channels could be investigated. It was shown that both dopamine and cyclic AMP decreased the open probability of horizontal cell gap junction channels by decreasing their open duration (McMahon, Knapp and Dowling 1989).

Other studies, using conventional and patch recording techniques, from isolated horizontal cells revealed that dopamine, cyclic AMP and the catalytic subunit of cyclic AMP-dependent protein kinase, modulate a glutamate-gated conductance (Knapp and Dowling 1987; Liman, Knapp and Dowling 1989). Dopamine and cyclic AMP increased the sensitivity of the horizontal cell to kainate (a glutamate receptor agonist). In the intact retina, this would result in the depolarization of the horizontal cell in the dark by increasing the glutamate-gated current. The horizontal cell would also be less responsive to light because the increased sensitivity to glutamate means that a much greater decrease in glutamate is

needed to produce the same degree of voltage change observed prior to dopamine. The increased conductance also means that membrane resistance will be decreased explaining the reduced response of horizontal cell to full-field illumination after dopamine.

3.9 Functional roles of dopamine in the teleost retina

The preceding section reviewed the evidence demonstrating that dopamine can reduce the receptive-field size of horizontal cells. The following section will review investigations that have indicated a possible role for dopamine in the control of retinal processes other than horizontal cell receptive-field size. As well, information concerning the synaptic inputs to and activity of dopaminergic interplexiform cells will be summarized.

Many of these studies address the question of when dopamine is released in the fish retina. Studies of horizontal cell receptive-field size suggest increased dopamine release after prolonged darkness and there is some support for this conclusion from studies of dopaminergic interplexiform cells. However, other dopamine-dependent retinal processes indicate a correlation with light-adaptation and measurements of dopamine release in the retina also argue for light-induced release of dopamine.

It is not possible to resolve the controversy concerning when dopamine is released in the retina. Although the data indicating dopamine release in the light are

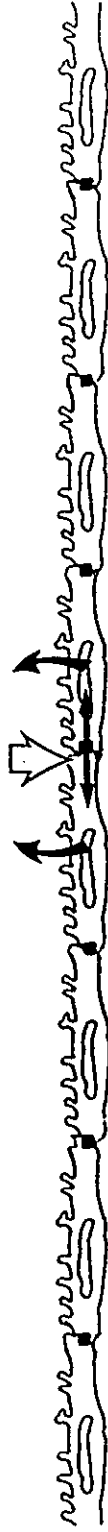
compelling, the results of such studies are equivocal. At the end of this section, the controversy will be summarized and briefly discussed.

3.10 Correlation of dopamine and darkness

By comparing the effects of prolonged darkness and dopamine on horizontal cells, it is apparent that the effects of both is to reduce receptive-field size and responsiveness. This correlation was first made by Mangel and Dowling (1985) who suggested dopamine is released in the dark. It followed, therefore, that the increase in the receptive-field size and responsiveness of horizontal cells by exposure of dark-adapted retinas to light was due to a decrease in dopamine release (Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988a, 1988b). These studies produced further support for the correlation of darkness and dopamine by showing that treatments that block dopamine either pharmacologically or by lesioning the endogenous source of dopamine, dopaminergic interplexiform cells, block the effects of darkness on horizontal cells. That is, darkness does not reduce the receptive-field size and responsiveness of horizontal cells in retinas treated with the selective D1 dopamine receptor antagonist SCH-23390 or lesioned with 6-hydroxydopamine. The effect of darkness, light and dopamine on horizontal cells summarized from these early observations is shown in Fig. 5.

Figure 5 Summary diagram of the effect of darkness, dopamine and light on horizontal cell receptive-field size based on early observations. Dopamine decreased the conductance of gap junctions (filled squares) and increased the conductance across the membrane (large arrows). As a result, the current generated by photoreceptor input (large arrow) does not pass to neighbouring horizontal cells and easily leaks out through the membrane. The consequence of this is a small receptive-field size and reduced responsiveness. Horizontal cells from dark-adapted retinas have similar physiological properties as in dopamine-treated retinas leading to the suggestion that dopamine is released onto horizontal cells in the dark. In the light, horizontal cell responsiveness and receptive-field size is increased presumably due to a decrease in retinal dopamine release resulting in increased gap junction conductance (open squares) and reduced membrane conductance (small arrows).

Prolonged Dark or Dopamine



Light



3.11 Effect of light and dopamine on other retinal features

There is evidence that dopamine regulates a variety of processes of the retina other than horizontal cell responsiveness and receptive-field size. In each case the action of dopamine mimics the effect of light on these retinal processes. A summary diagram illustrating the effect of dopamine and light on each of these processes is presented in Fig. 6.

3.12 Horizontal cell chromatic responses

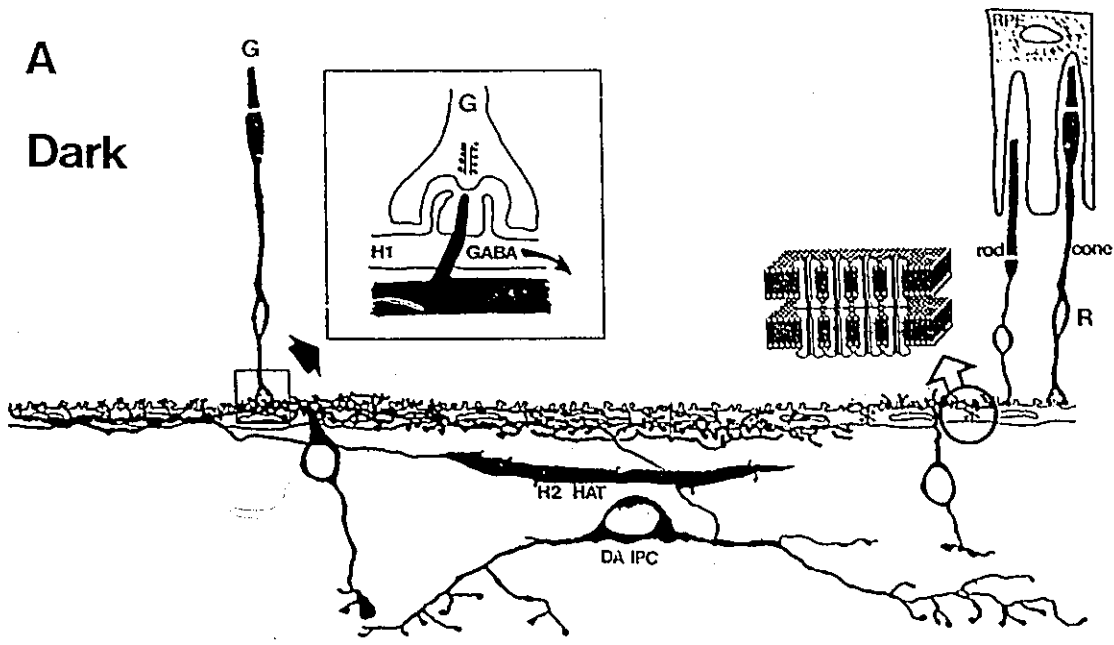
The pattern of fish horizontal cell response to chromatic stimuli has been shown to depend on the state of retinal adaptation. This was first reported by Weiler and Wagner (1984) who found that the depolarizing responses to red stimuli of H2 horizontal cells was present only in moderately or completely light-adapted carp retinas. This result has been confirmed by Djamgoz et al. (1988) in the roach.

Djamgoz, Kirsch and Wagner (1989) have shown that the appearance of depolarizing responses of H2 horizontal cells in light-adapted retinas can be blocked by the dopamine receptor antagonist (D2>D1) haloperidol. This suggests that the effect of light-adaptation on H2 horizontal cell chromatic responses may be mediated by the release of dopamine.

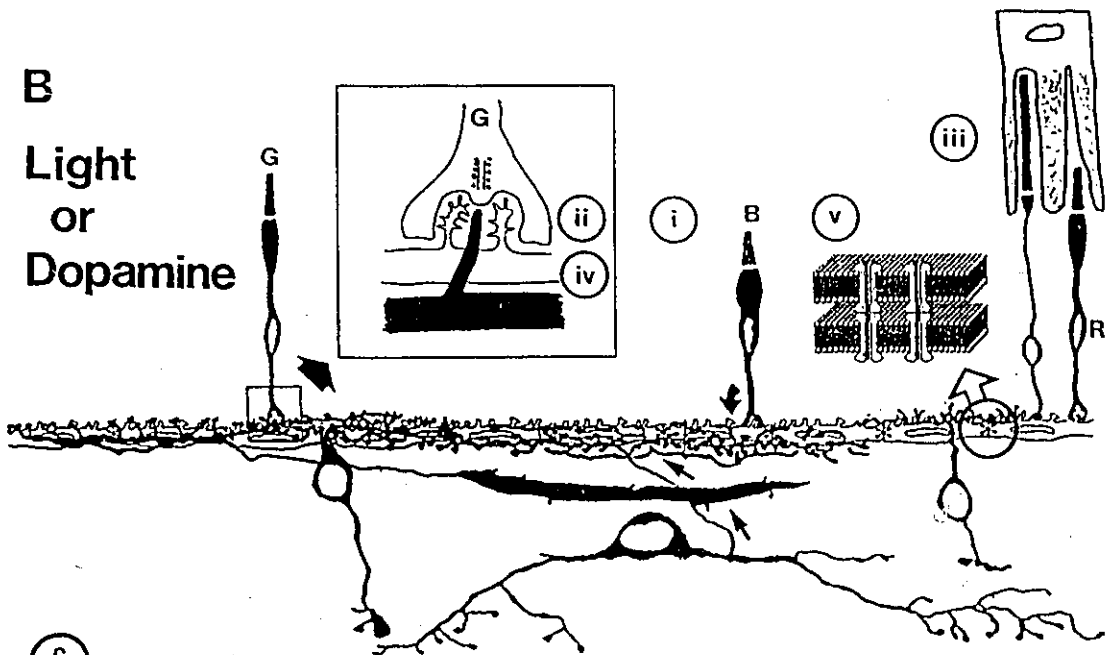
It has been suggested there may be more than one type of neurotransmitter input to H1 horizontal cells with the blue cone using a transmitter other than glutamate (see Section 2.17). The strength of the short-wavelength (blue) input to

Figure 6 Effects of light and dopamine on the retina. After light-adaptation or dopamine treatment (B) of a dark-adapted retina (A), (i) blue cone input to H1 horizontal cells become apparent, (ii) horizontal spinules form, (iii) rods elongate, cones contract and retinal pigmented epithelium pigment disperses, (iv) GABA release from H1 horizontal cells is decreased and (v) horizontal cell gap junction connexon density is decreased. B = blue cone, G = green cone, R = red cone, RPE = retinal pigmented epithelium, H2 HAT = H2 horizontal cell axon terminal. Figures of some of the cells based on drawings by R.E. Marc. Illustration of gap junction connexons modified from Kandel and Siegelbaum (1985).

A
Dark



B
Light
or
Dopamine



H1 horizontal cells in carp is reduced following long-wavelength, but not short-wavelength, adaptation (Djamgoz and Yamada 1992). The effect of long-wavelength illumination was mimicked by dopamine (Yasui, Yamada and Djamgoz 1990; Djamgoz and Yamada 1992) suggesting that light-stimulated release of dopamine can modulate the chromatic inputs to H1 horizontal cells.

3.13 Horizontal cell gap junction morphology

Differences in the morphology of gap junctions, visualized by freeze-fracture electron microscopy, are seen in dark- vs. light-adapted retinas (Wolburg and Kurz-Isler 1985; Baldrige, Miller and Ball 1987; Washioka et al. 1992). The connexon density of gap junctions on horizontal cell somata are increased in dark-adapted retinas compared to light-adapted retinas. Although Wolburg and Kurz-Isler (1985) and Washioka et al. (1992) report similar changes in horizontal cell axon terminal gap junctions, Baldrige, Ball and Miller (1987) did not. Qian and Ripps (1992) studied the morphology of rod horizontal cell gap junctions in the all-rod retina of the skate but found no difference between light- and dark-adapted retinas. However, no change in receptive-field size was detected either.

The effect of light on horizontal cell gap junctions was mimicked by intraocular injections of dopamine (Baldrige, Ball and Miller 1987; Washioka et al. 1992) and both were blocked by the dopamine receptor (D2>D1) antagonist

haloperidol. To determine if changes in horizontal cell gap junction morphology could be related to the activity of dopaminergic interplexiform cells, Baldrige, Miller and Ball (1989) studied the effect of light and dopamine in retinas previously lesioned with 6-hydroxydopamine. Regardless of the adaptation state, lesioned retinas had gap junction morphology similar to that of unlesioned dark-adapted retinas. Dopamine reduced gap junction connexon density in lesioned retinas but light did not. Similar results have been reported by Washioka et al. (1992). These results suggest that light stimulated release of dopamine *in vivo* could affect the morphology of horizontal cell gap junctions. Curiously, Schmitz and Wolburg (1991) reported that they were unable to detect significant differences in horizontal cell gap junction connexon density after dopamine or haloperidol treatment.

3.14 Horizontal cell spinules

Raynauld, Laviolette and Wagner (1979) first noticed that the lateral horizontal cell elements within the cone terminal of goldfish retina had finger-like extensions with membrane densities at the tips. These structures, called "spinules" (Wagner 1980), are apparently unique to fish (Raynauld, Laviolette and Wagner 1979) and are present in greater numbers in light-adapted retinas than in dark-adapted retinas. It has subsequently been suggested that the light-dependent change in the chromatic responses of H2 horizontal cells are due to the presence of spinules in the light (Weiler

and Wagner 1984). There is further speculation that spinules may be the site of horizontal cell to cone feedback (Weiler and Wagner 1984) and, therefore, a source of the receptive field surround of ganglion cells (Raynauld, Laviolette and Wagner 1979).

It has been suggested that the presence of the depolarizing responses of H2 horizontal cells to long wavelength stimuli is controlled by the release of dopamine in the retina (Djamgoz, Kirsch and Wagner 1989). Since it has also been suggested that the morphological pathway of the depolarizing responses is via spinules (Weiler and Wagner 1984) it follows that spinule formation should also be under dopaminergic control. Dopamine has been shown to mimic the effect of light by inducing spinule formation in dark-adapted carp retina (Weiler et al. 1988a). Haloperidol blocked the effect of dopamine and reduced the expression of spinules in light-adapted retina (Weiler et al. 1988a) as did 6-hydroxydopamine lesioning (Kirsch, Wagner and Djamgoz 1991; Kohler and Weiler 1990).

There is disagreement regarding whether the effect of dopamine on horizontal cell spinule formation depends on the same mechanism (cyclic AMP production) that mediates horizontal cell receptive-field size. Kohler and Weiler (1990) showed that spinule formation was not induced by intraocular injection of cyclic AMP analogues, adenylate cyclase stimulators or phosphodiesterase inhibitors. However,

Behrens et al. (1992) report that increasing intracellular levels of cyclic AMP in the isolated fish (roach) retina did induce spinule formation.

Nonetheless, Weiler, Kohler and Janssen (1991) demonstrated that spinule formation could be induced by activating protein kinase C (PKC) and that light-dependent formation of spinules was blocked by the PKC inhibitor, sphingosine. Although there is some evidence that activators of PKC can stimulate dopamine release in the fish retina (Kato et al. 1990) the effects of PKC on spinules does not appear to be dopamine-mediated since the effects of PKC-related drugs were not abolished by 6-hydroxydopamine lesioning (Weiler, Kohler and Janssen 1991).

In isolated horizontal cells, dopamine, phorbol esters and synthetic diacylglycerol analogues, but not cyclic AMP, induced neurite retraction, effects blocked by the PKC inhibitor staurosporine (Rodrigues and Dowling 1990). These results suggest that dopamine causes neurite retraction by the activation of PKC via diacylglycerol. It is not yet clear if the horizontal cell neurite extension *in vitro* is equivalent to spinule formation observed within the cone synaptic terminal, despite the fact that the mechanism controlling both appear identical. However, this possibility seems unlikely because the effects of dopamine on spinule formation and neurite retraction of isolated cells are opposite. These results may indicate there are at least two intracellular mechanisms of

horizontal cells that change in response to dopamine: cyclic AMP and PKC. Unfortunately, PKC has not been localized immunocytochemically to horizontal cells in teleosts but has been found in bipolar cells and dopaminergic interplexiform cells (Negishi, Kato and Teranishi 1988; Susuki and Kaneko 1990).

3.15 Retinomotor movements

The refractive index of the cornea and aqueous humour is very near that of water making the lens the only refractive element in the fish eye (Walls 1942). As a consequence, the lens of fishes are spherical and quite large relative to the size of the eye. In fact, the lens often protrudes through the pupil. It may be for this reason that the pupil of most fishes is immobile (Walls 1942).

Instead of controlling the amount of light entering the eye with a pupil, fishes control the amount of light incident on the photoreceptors at the level of the photoreceptor-pigmented epithelium interface. In the light, cone myoids contract, rod myoids elongate, and retinal pigmented epithelium pigment granules disperse. In the dark, cones elongate and rods contract while pigment aggregates to the basal portion of the retinal pigmented epithelium. These changes are often described by the term "retinomotor movements" (Ali, 1975). In addition to being responsive to changes in light, the movement of cones is subject to an

endogenous circadian rhythm (Douglas and Wagner 1982; Burnside and Ackland 1984; McCormack and Burnside 1991a, 1991b).

Dopamine mimics the effect of light-adaptation on retinomotor movements in the green sunfish acting via D2 dopamine receptors (Dearry and Burnside 1986a). Compounds that promote the release of dopamine, such as GABA receptor antagonists or serotonin, mimic the effect of dopamine and light on retinomotor movements (Dearry and Burnside 1986b). These results are consistent with light-induced retinomotor movements controlled by dopamine release.

It is still not clear how dopamine, released at the level of horizontal cells, might control retinomotor movements. Dopaminergic interplexiform cells rarely make synaptic contacts with photoreceptors and have never been shown to extend as far as the retinal pigmented epithelium. Therefore, if dopamine modulates retinomotor movement, dopamine released from interplexiform cell processes at the level of the horizontal cells must diffuse to reach receptors on photoreceptors and pigmented epithelium. A precedent for such diffusion of dopamine comes from studies in animals where horizontal cell receptive-field size is regulated by dopamine, but dopamine is contained in amacrine cells rather than interplexiform cells (Piccolino and Demontis 1988).

Dopaminergic regulation of retinomotor movements has recently been questioned by two studies that investigated the effect of 6-hydroxydopamine lesioning on retinomotor movements

(Douglas et al. 1992; Ball, Baldrige and Fernback 1993). These studies showed that the light, dark and circadian components of retinomotor movements were unaffected by 6-hydroxydopamine lesioning. On the basis of these results it is tempting to conclude that dopamine is not important in the control of retinomotor movements. However, an alternate explanation is there is at least one mechanism, in addition to dopamine, that can control retinomotor movements. Whether this mechanism is present in normal unlesioned retinas or becomes apparent only after lesioning is unknown. One possible alternate regulator to dopamine is an adrenergic agent that could act on α -adrenergic receptors also implicated in the control of retinomotor movements (Deary and Burnside 1988).

A possible explanation for the apparent discrepancy between the effects of light and dopamine on retinomotor movements versus horizontal cell receptive-field size are major differences in the experimental protocol. In studies of retinomotor movements using isolated retinas (Deary and Burnside 1986a, 1986b) 5.0 mM taurine was always added to the superfusate-Ringer solution. The argument used to justify the addition of this amino acid was that retinas of mice, rats and chicks have the highest levels of taurine in the central nervous system (Pasantes-Morales et al. 1972; Cohen, McDaniel and Orr 1973). In the absence of 5.0 mM taurine, prolonged darkness caused cones to contract as in light-adapted retinas

(Dearry and Burnside 1984). Thus without taurine prolonged dark-adaptation is mimicked by the effect of dopamine on short term dark-adapted retinas. This is exactly what is observed from studies of horizontal cell receptive-field size. The functional consequences of the addition of 5.0 mM taurine to the Ringer are completely unknown.

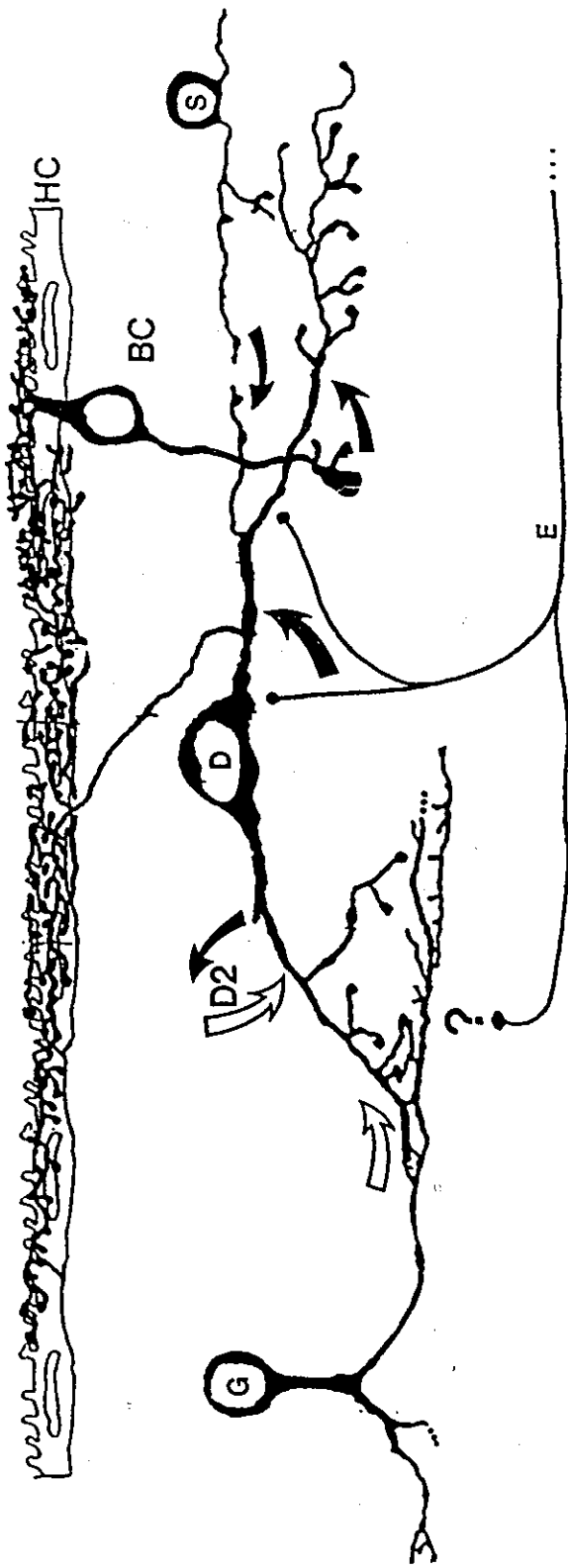
3.16 Inputs to the dopaminergic interplexiform cell

The control of dopamine release in the teleost retina has been demonstrated to be influenced by at least four neurotransmitter systems: GABA, serotonin, enkephalin and gonadotropin hormone-releasing hormone. A summary diagram of these inputs is presented in Fig. 7.

3.17 GABA

The GABA antagonist bicuculline mimics the effect of dopamine on horizontal cells in both turtle and carp retina (Piccolino et al. 1982; Negishi, Teranishi and Kato 1983). Because the effect of bicuculline on horizontal cells was blocked by dopamine receptor antagonists and was abolished after lesioning with 6-hydroxydopamine (Piccolino et al. 1982; Cohen and Dowling 1983; Negishi et al. 1983) it has been suggested that dopaminergic neurons receive an inhibitory GABAergic innervation. This suggestion was supported further by studies that demonstrated an increased release of pre-loaded [³H]-dopamine in the presence of bicuculline and a decrease in the presence of GABA or the GABA agonist muscimol (O'Connor et al. 1986, O'Connor, Zucker and Dowling 1987;

Figure 7 Summary diagram of inputs to the dopaminergic interplexiform cell. The dopaminergic interplexiform cell (D) receives excitatory input from serotonergic amacrine cells (S), ON bipolar cells (BC) and efferent fibres (E) (solid arrows). Inhibitory input comes from GABAergic amacrine cells (open arrow). Efferent fibres contact a variety of other retinal neurons, including GABAergic amacrine cells, but whether such contacts are excitatory or inhibitory is not known. Release of dopamine is also subject to D2 receptor regulation which may indicate a D2 autoreceptor on the processes or somata of the dopaminergic interplexiform cell. The major target of dopaminergic interplexiform cells, the horizontal cells (HC), are also depicted. Figures of cells are based on drawings by R.E. Marc.



Ishita et al. 1988). Using retinomotor movements as an indicator of dopamine release, Dearry and Burnside (1986b) also provided evidence suggesting the existence of an inhibitory GABAergic input to dopaminergic interplexiform cell of the fish retina.

There is also anatomical evidence for GABAergic input to dopaminergic interplexiform cells. Yazulla and Zucker (1988) demonstrated synaptic contacts between dopaminergic interplexiform cell processes, labelled by tyrosine hydroxylase immunocytochemistry, and GABAergic amacrine cells using [³H]-GABA uptake. The origin of the GABAergic amacrine cell processes is most likely from pyriform Ab amacrine cells or displaced amacrine cells (Yazulla and Zucker 1988).

3.18 Serotonin

Serotonin and serotonin agonists have been reported to increase the release of pre-loaded [³H]-dopamine from fish retina (Kato et al. 1982) and decrease retinal stores of dopamine (Jaffe et al. 1987) in a calcium-dependent manner. Another study, however, reported that serotonin-induced [³H]-dopamine release was calcium independent (O'Connor et al. 1986). Indoleamine-accumulating and serotonin-immunoreactive amacrine cells have been described in the teleost retina (Ehinger and Floren 1978; Marc 1982). Therefore, serotonin-induced dopamine release may indicate that the dopaminergic interplexiform cell receives an excitatory input from serotonergic amacrine cells. The possible contact of putative

serotonergic cells with dopaminergic interplexiform cells comes from evidence that 1) the putative serotonergic amacrine cells arborize primarily in sublayer 1 of the inner plexiform layer (Ehinger and Floren 1978; Marc 1982), where processes of dopaminergic interplexiform cells are found, and 2) that the indoleamine cells appear to be paired with dopaminergic interplexiform cells in the carp retina (Negishi, Kato and Teranishi 1981). However, one electron microscope study reports the absence of any contacts of indoleamine cells on dopaminergic interplexiform cells or processes in the carp retina (Holmgren-Taylor 1983).

3.19 Efferent fibre transmitters

Ramón y Cajal (1892) described fibres in the optic nerve fibre layer in amphibian, reptilian, avian and mammalian retinas that ramified in the inner plexiform and inner nuclear layers. It was ultimately shown that many species of fish also possess such centrifugal fibres (Witkovsky 1971; Ebbesson and Meyer 1981). Although there may be multiple sources of efferent fibres that project from the brain to the retina in fish (Ebbesson and Meyer 1981), there is agreement that one source of retinal efferents is the terminal nerve (Springer 1983). The somata of the terminal nerve are found along its course on the ventromedial surface of the olfactory bulb and project to the olfactory epithelium, medial and lateral septal nuclei, and olfactory tubercle (Sheldon 1909; Brookover 1910).

In teleosts, the neurons and processes of this olfactoretinal system contain two peptides: gonadotropin hormone-releasing hormone (GnRH) and molluscan cardioexcitatory peptide (FRMFamide) (Stell et al. 1984). Zucker and Dowling (1987) demonstrated contacts made by these efferent fibres onto dopaminergic interplexiform cells and ultimately Umino and Dowling (1991) showed that GnRH induced the release of dopamine onto horizontal cells, however that study did not reach any definitive conclusions regarding the effect of FRMFamide. The idea that centrifugal input to the retina might be important to the function of horizontal cells was also suggested by studies that showed changes in gap junction morphology after optic nerve crush (Wolburg and Kurz-Isler 1985). It is possible, therefore, that the efferent projections from the olfactory system to the retina represent some form of olfactory-directed modulation of visual sensitivity related to dopaminergic control of horizontal cell function.

The emphasis placed on the efferent fibre input to dopaminergic interplexiform cells by Zucker and Dowling (1987) has been questioned by the study of Ball, Stell and Tutton (1989) that showed significant contacts of GnRH-immunoreactive fibres not only with dopamine-accumulating processes but also with glycine- and GABA-accumulating processes in the inner plexiform layer. Furthermore, the effects of GnRH on ganglion cell activity (Walker and Stell 1986) did not reveal changes

that could be specifically associated with changes in horizontal cell activity. Therefore, while the efferent fibre system may modulate dopaminergic interplexiform cells, it is likely that this system has a wider functional role by affecting the activity of a variety of retinal neurons.

3.20 Protein kinase C

Negishi, Kato and Teranishi (1988) showed that dopaminergic neurons of the carp and goldfish retina are immunoreactive to the α and β forms of protein kinase C (PKC). This led Kato et al. (1990) to determine if PKC-related drugs had any effect on dopamine release in the carp retina. They reported that a phorbol ester and synthetic diacylglycerol both stimulated the release of dopamine from isolated retina, effects that were blocked by PKC inhibitors. The significance of these findings is not yet clear but may suggest that one of the neurotransmitters that regulate dopamine release in the retina may do so by interacting with phosphatidylinositol turnover.

3.21 D2 dopamine receptor regulation of dopamine release

Many dopaminergic neurons of the central nervous system possess pre-synaptic D2 dopamine receptors that act as feedback loops controlling the magnitude of dopamine release (for review see Starke, Gothert and Kilbinger 1989). There is now evidence for similar D2 receptor-mediated regulation of dopamine release in the fish retina. Harsanyi and Mangel (1992) have shown that agonists of D2 receptors increase the

receptive-field size of goldfish horizontal cells presumably by decreasing dopamine release. The results of Rashid, Baldrige and Ball (1993) support these findings by demonstrating that D2 receptor agonists decrease [³H]-dopamine release from goldfish retina.

3.22 Other inputs to dopaminergic interplexiform cells

Yazulla and Zucker (1988) reported that 4% of the inputs to dopaminergic interplexiform cells came from ON bipolar cells. Yazulla and Zucker (1988) also observed unidentified amacrine cell contacts that might represent input from cholinergic amacrine cells (Negishi and Drujan 1979; Hayashi 1980).

3.23 Measures of dopamine release in the teleost retina

There are only three studies in the literature that report light-dependent changes in dopamine release from teleost retina. The first (Kirsch and Wagner 1989) reported an increase in dopamine release in the light, compared to the dark. However, the stimulus used was a flickering light making it unclear which of the two components of the flickering light (onset or offset) induced the change in dopamine release. The second study (Weiler, Kolbinger and Kohler 1989) demonstrated a gradual decrease of dopamine release of a light-adapted retina placed in the dark. However, light-stimulated release of dopamine was not demonstrated. The third study (Dearry and Burnside 1989) showed higher levels of dopamine in the medium surrounding

retinas incubated in the light than surrounding retinas incubated in the dark. However, the solution in which the retinas were incubated contained taurine raising questions of what effect this amino acid alone might have on dopamine release. Although each study suggests greater release of dopamine in the light, each study is not unequivocal.

Other indirect measures of dopamine release also suggest light-stimulated release of dopamine in the teleost retina (Weiler, Kolbinger and Kohler 1989; Kolbinger et al. 1990). Levels of dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite, are decreased in the dark and increased during constant illumination suggesting greater turnover of dopamine in the light. Both dopamine release induced by increasing extracellular potassium and retinal dopamine content are decreased in the light compared to the dark suggesting reduced cellular stores of dopamine as a consequence of greater dopamine release in the light.

3.24 Tyrosine hydroxylase activity

It has been reported that the activity of tyrosine hydroxylase, the rate-limiting enzyme in the production of dopamine, is increased by light in the fish retina (Dearry 1991). However, a more recent study of tyrosine hydroxylase activity over a 24 hr light:dark cycle clearly shows increased activity in the dark (McCormack and Burnside 1993). It is not clear, however, that increased tyrosine hydroxylase activity necessarily translates into increased dopamine release.

3.25 Intracellular recordings from dopaminergic interplexiform cells

There are no studies of the effect of light on [³H]-dopamine release in the fish retina. However, studies have clearly established that [³H]-dopamine release from the fish retina is calcium-dependent (Kato et al. 1982; Jaffe et al. 1987; O'Connor et al. 1986; Rashid, Baldrige and Ball, 1993). Therefore, one might expect that dopamine release would occur when dopaminergic interplexiform cells are depolarized (Katz and Miledi 1967). Unfortunately, intracellular recordings from dopaminergic interplexiform cells are extremely rare and uncertain. In two reports, one from the dace retina (Hashimoto, Abe and Inokuchi 1980) and one from goldfish (Djamgoz, Usai and Vallerga 1991), presumed dopaminergic interplexiform cells responded with ON, OFF or transient ON-OFF responses to light. It is not clear, therefore, which stimulus would result in the greater release of dopamine. However, Djamgoz, Usai and Vallerga (1991) also showed that a presumed dopaminergic interplexiform cell had oscillatory potentials in the dark. These oscillations were absent during illumination. Therefore, the continued cycles of depolarization in the dark may represent optimal conditions for dopamine release.

3.26 Controversy: When is dopamine released in the teleost retina?

It is apparent that the studies of horizontal cell receptive-field size and responsiveness, which argue for greater release of dopamine in the dark, and the studies of dopamine release, which argue for greater release in the light, do not agree. Furthermore, studies of the activity of other apparently dopamine-mediated events also suggest light-induced release. These differences are summarized in Table 3.

Correlating the lighting condition with the effect of exogenous dopamine application on a feature of retinal processing does not directly prove that dopamine is the mediator of the light signal *in vivo*. However, demonstrating that the effect of light or dark can be blocked with a dopamine receptor antagonist provides further evidence supporting the involvement of a dopaminergic mechanism. The finding that so many retinal features thought to be controlled by light are blocked by dopamine antagonists appears to present a substantial case for light-stimulated release of dopamine. It is unclear why retinomotor movements, which are blocked by D2 antagonists, are not abolished by 6-hydroxydopamine lesioning (Table 3) (Douglas et al. 1992; Ball, Baldrige and Fernback 1993).

The strongest evidence supporting light-stimulated release of dopamine are in studies where release was directly measured. However, it is unclear where the measured dopamine

Table 3--Effects of dopamine on the teleost retina.

	dopamine correlates with adaptation to:	adaptation blocked by a dopamine receptor antagonist?	adaptation abolished by 6-OHDA lesioning?
horizontal cell receptive-field size	dark	yes	yes
chromatic responses of horizontal cells	light	yes	not done
horizontal cell gap junction morphology	light	yes	yes
horizontal cell spinule formation	light	yes	yes
retinomotor movements	light	yes	no

is actually released in the retina. Since dopaminergic interplexiform cells ramify both in the inner and outer plexiform layer, it is possible that dopamine released in the inner plexiform layer accounts for most of the dopamine release into the superfusate in the light. There is, however, no way to distinguish between the release of dopamine in the inner and outer plexiform layer leaving the idea that dopamine is released differentially from processes of the same cell as speculative.

Although several neurotransmitter systems have been implicated in the control of dopamine release, little is known about the response properties of these neurons to light and dark. The input to the dopaminergic interplexiform cell from the ON bipolar cell, however, suggests a light-dependent release of dopamine but these constitute a small percentage of the total input to the dopamine cell. It is not possible to make a prediction on the behaviour of the dopaminergic interplexiform cell from the response properties of the neurons which provide input to it. Although there is some suggestion that dopaminergic interplexiform cells depolarize in the dark (Djamgoz, Usai and Vallergera 1991) the sample of interplexiform cells from which such recordings have been made is far too small to be accepted with certainty.

Weiler, Kolbinger and Kohler (1989) point out that it may not be possible to associate changes in horizontal cell responses in light- or dark-adapted retinas with changes in

dopamine release because of technical difficulties. Because a dim, but visible, red light is used to dissect the retina and align the electrode in electrophysiological experiments, the retina may not be actually dark-adapted.

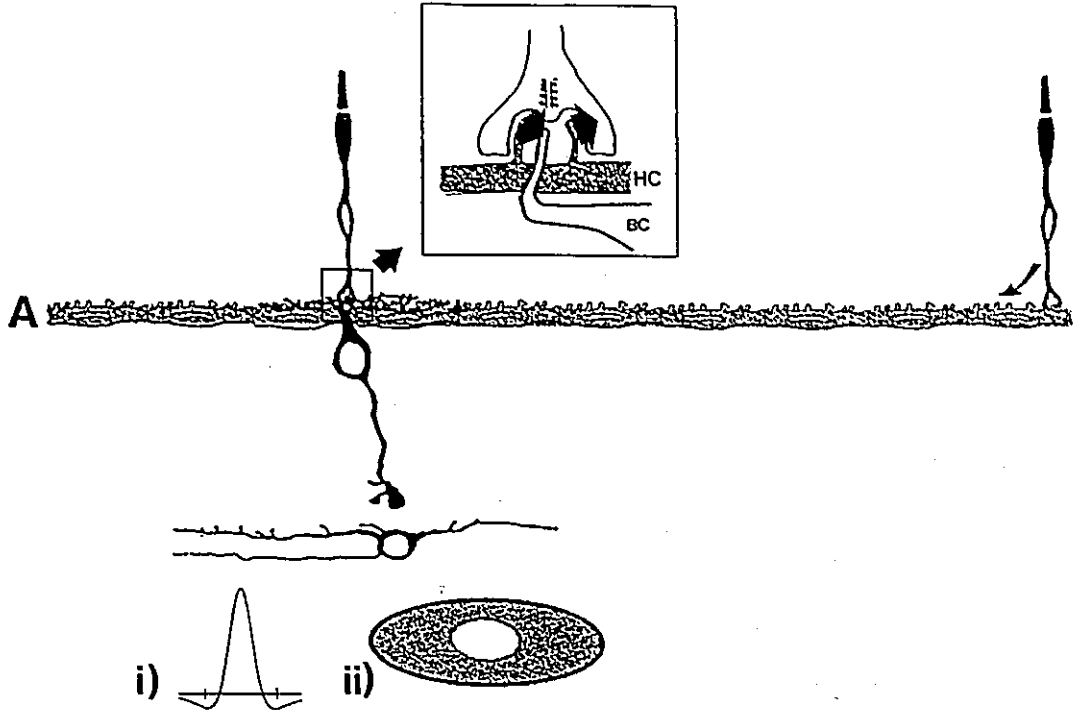
3.27 Adaptation and the role of horizontal cells in the retina

Horizontal cells provide the surround component of bipolar cells and therefore ultimately contribute to the receptive-field surround of ganglion cell. It has been suggested that the reduced responsiveness and uncoupling of horizontal cells in the dark is consistent with the reduction of ganglion cell receptive fields (Mangel and Dowling 1985; Dowling 1987; Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988a, 1988b). Retinal function becomes more concerned with sensitivity than acuity as light levels fall (Barlow, Fitzhugh and Kuffler 1957; Raynauld, Laviolette and Wagner 1979; Dowling 1987). One way to accomplish this is to remove the inhibitory influence of horizontal cells on bipolar cells and cones. A summary diagram of the effect of darkness on horizontal cell input to bipolar cell and ganglion cell receptive-field surround is presented in Fig. 8.

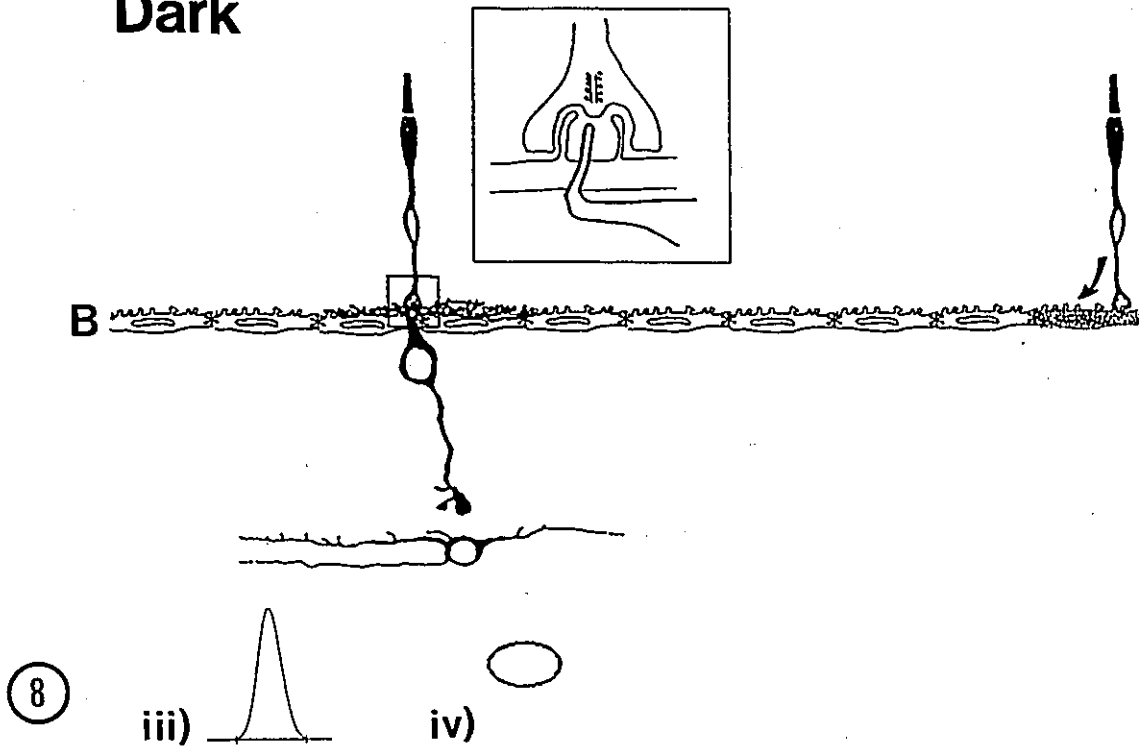
There is recent evidence suggesting that carp rod horizontal cells may also parallel cone horizontal cells by changing sensitivity and receptive-field size with the level of ambient illumination, but over a dimmer range of light intensities than cone horizontal cells (Villa, Bedmar and Barón 1991). It remains to be determined if rod horizontal

Figure 8 Effects of light and dark on horizontal cell receptive-field size and formation of bipolar and ganglion cell receptive field surrounds. (A) In a light-adapted retina, horizontal cell receptive-field size is increased so cones distant from a given bipolar cell have an effect on other cones (feedback) or bipolar cells (feedforward). The heavy shading of the horizontal cells illustrate the effect the cone on the right has on the cone and bipolar cell on the left. Under these conditions the antagonistic centre-surround receptive-field organization of ganglion cells is present as depicted by Gaussian curve (i), or by cartoon (ii). (B) In the dark-adapted retina, horizontal cell receptive-field size decreases, as illustrated by limiting the shading of horizontal cells to one cell. The input from one cone to the other cone and bipolar cell via horizontal cells is lost. Thus, the inhibitory surround of the ganglion cell is lost (iii), (iv). This will increase the overall sensitivity of the ganglion cell but does so at the expense of spatial acuity (also see Fig. 3).

Light



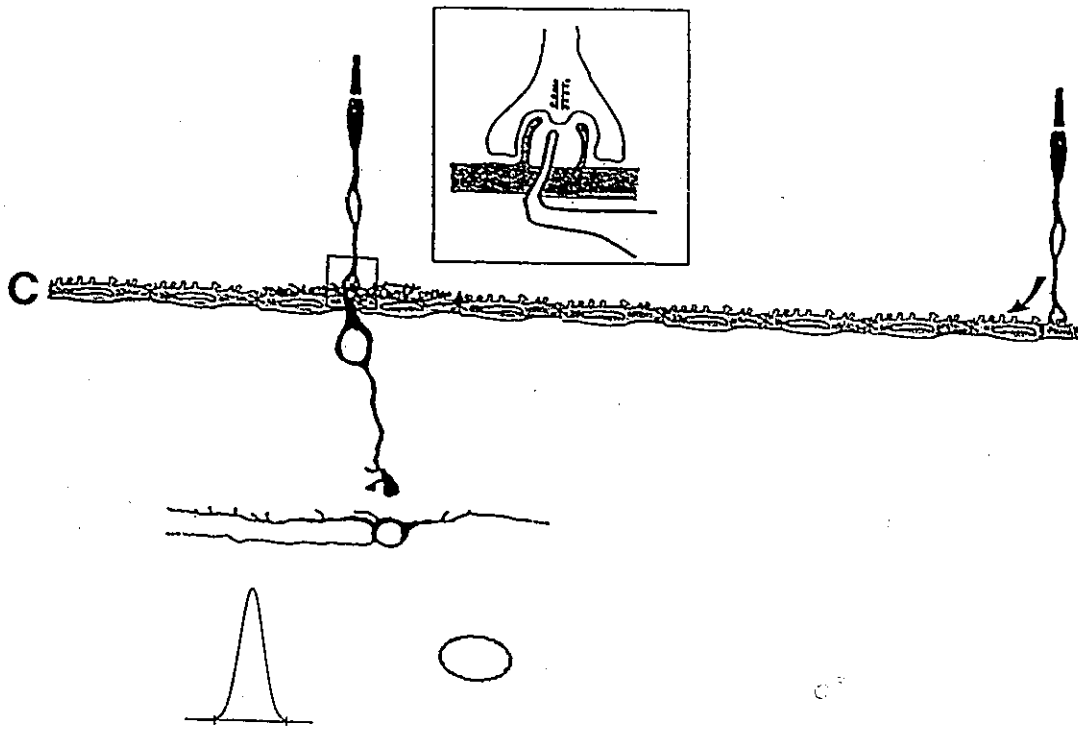
Dark



8

(C) An alternate model that could explain the observed loss of the ganglion cell surround is that horizontal cell coupling increases thereby reducing input resistance. This is indicated by the broad but light shading of the horizontal cell in the diagram. As long as the change in photoreceptor transmitter is kept limited (as in dim light) the change will be insufficient to affect horizontal cell potential markedly and, therefore, there will be little or no feedback or feedforward within the synaptic terminal. Figures of cells based on drawings by R.E. Marc.

Dark



cells contribute to the receptive-field surround of bipolar cells in the teleost retina since the only evidence that it does comes from an all-rod elasmobranch retina (Naka and Witkovsky 1972).

Although the loss of horizontal cell input to cones and bipolar cells by uncoupling is compelling, it is not necessarily the case that horizontal cells must be uncoupled to remove the inhibitory input from cones and bipolar cells. It is conceivable that increased horizontal cell coupling could effectively abolish the inhibitory input to cones and bipolar cells under dim light conditions. This is summarized in Fig. 8C. Therefore, it is not necessary to conclude that horizontal cells must be uncoupled in the dark to satisfy the condition that the ganglion cell receptive field surround is reduced in the dark.

4.0 Intent of thesis work

The first objective of the thesis work was to investigate further the receptive-field properties of horizontal cells after different adaptation conditions. There is considerable evidence that suggests dopamine is released in the light. In Chapter II, the effect of background light on the horizontal cell receptive-field size of dark-adapted goldfish retina was studied to determine if there was any change in horizontal cell receptive-field size due to light that might be linked to dopamine release. These studies showed that the receptive-field size of horizontal cells is

decreased by light, but occurs by a non-dopaminergic mechanism. In Chapter III the effect of background light will be examined further to illustrate that the fish retina cannot be classified simply as light- or dark-adapted on the basis of horizontal cell receptive-field size. I conclude that it is important to distinguish between dark-adapted, light-sensitized and light-adapted retinas. Additional evidence supporting dark-release of dopamine will also be discussed.

Having established that light reduces horizontal cell receptive-field size by a non-dopaminergic mechanism, the remainder of the thesis attempts to understand the mechanism underlying this light-dependent reduction. First, in Chapter IV and V, two possible sources of adrenergic input to horizontal cells are explored but found to be unsuitable candidates for the mediator of the light-dependent reduction of horizontal cell receptive-field size. In Chapter VI evidence will be presented suggesting that nitric oxide may be involved in the light-dependent reduction of horizontal cell receptive-field size.

The thesis shall be concluded by a discussion in Chapter VII that relates the significance of the thesis work to the controversy of dopamine release in the fish retina and to the role of horizontal cells in visual processing. This discussion shall include a brief comparison of the fish retina with other vertebrate retinas as well as an overview of the importance of this work to the understanding of human vision.

II. Background illumination reduces horizontal cell
receptive-field size in both normal and 6-hydroxydopamine-
lesioned goldfish retinas

1.0 Preface and significance to thesis

This work was presented at the 1990 Association for Research in Vision and Ophthalmology meeting and was subsequently published (Baldrige and Ball 1991).

This study shows that background light reduced the receptive-field size of goldfish horizontal cells partly by increasing gap junction resistance. It was demonstrated that this change was not due to the release of dopamine from interplexiform cells.

The responsiveness and receptive-field size of horizontal cells have been reported to decrease in the dark and increase in the presence of light, a process called "light-sensitization." The retinas used in the present study were similar to the "light-sensitized" state at the beginning of these experiments. The finding that light reduced the receptive-field size of horizontal cells in a "light-sensitized" retina suggests there are at least two conditions where horizontal cell receptive-field size is decreased: dark-adapted and light-adapted. Between these two conditions is the "light-sensitized" condition, where horizontal cells

have the greatest responsiveness and have an increased receptive-field size (see Preface Fig. 1, page 91).

The finding that dopamine was not the mediator of the light-dependent change in horizontal cell receptive-field size suggests that dopamine is not released in the light in the fish retina. These results suggest, therefore, there must be some other mechanism in the retina by which horizontal cell receptive-field size, and specifically horizontal cell coupling, can be controlled.

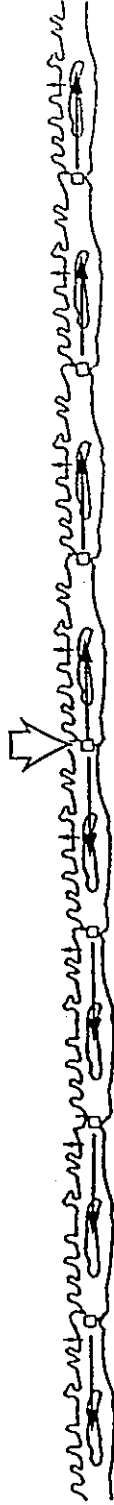
Subsequently, another study, in the white perch retina, came to similar conclusions (Umino, Lee and Dowling 1991).

Figure P1 Dark-adapted, light-sensitized and light-adapted horizontal cells. (Dark) In the dark receptive-field size and responsiveness of horizontal cells are reduced. This is because current resulting from photoreceptor input (open arrow) does not spread to adjacent horizontal cells because it cannot pass through gap junctions and readily leaks across the membrane. This is a consequence of increased gap junction resistance (filled squares) and decreased membrane resistance (filled arrows). The latter is a result of an increase in the sensitivity of a glutamate gated conductance (g_{Glu}) which also explains their reduced responsiveness. These changes are due to increased release of dopamine from interplexiform cells. (Light-sensitized) When a dark-adapted retina is exposed to an adapting light, dopamine release decreases. Therefore, gap junction resistance decreases and membrane resistance increases. As a result, current from photoreceptor input passes through gap junctions, does not leak across the membrane and the cell is less sensitive to glutamate. This means that the receptive-field size and responsiveness will increase. (Light-adapted) When a light-sensitized retina is exposed to even more light, the receptive-field size of horizontal cells decreases due to an increase in gap junction resistance. The mechanism of this change is unknown but is not related to increased dopamine release. Membrane resistance may also change but this has not yet been demonstrated.

Dark



Light-sensitized



Light-Adapted



(P1)

2.0 Abstract

The effect of background illumination on horizontal cell receptive-field size and dye coupling was investigated in isolated superfused goldfish retinas. Background illumination reduced both horizontal cell receptive-field size and dye coupling. The effect of light on horizontal cell receptive-field size was mimicked by treating the retina with 20 μ M dopamine. To test the hypothesis that the effects of light were due to endogenous dopamine release, the effect of light was studied in goldfish retinas in which dopaminergic interplexiform cells were lesioned using 6-hydroxydopamine treatment. In lesioned retinas, background illumination reduced both horizontal cell receptive-field size and dye coupling. Furthermore, the effect of background illumination on unlesioned animals could not be blocked by prior treatment with the D₁ dopamine receptor antagonist SCH-23390. These results suggest that, in goldfish retina, dopamine release is not the only mechanism by which horizontal cell receptive-field size could be reduced by light.

3.0 Introduction

Horizontal cells are second order neurons of the vertebrate retina which contribute to the receptive field surround of bipolar cells (Toyoda and Tonasaki 1978). The receptive-field size of horizontal cells is larger than their dendritic field size (Naka and Rushton 1968; Stell and Lightfoot 1975) due to extensive coupling by gap junctions (Stell 1972; Witkovsky, Burkhardt and Nagy 1979). Dopamine reduces the receptive-field size of horizontal cells (Teranishi, Negishi and Kato 1984) suggesting that dopamine may cause an increase in the resistance of gap junctions (Lasater and Dowling 1985; McMahon, Knapp and Dowling 1988), a decrease in horizontal cell membrane resistance (Knapp and Dowling 1987), or both. In fish, horizontal cells are contacted by dopaminergic 'I1' interplexiform cell processes (Dowling and Ehinger 1978) which could provide an endogenous source of dopamine capable of regulating the receptive-field size of horizontal cells in vivo.

There is disagreement as to the conditions under which dopamine is released in the fish retina. Several studies have demonstrated that the receptive-field size of horizontal cells is decreased after exposure to prolonged darkness compared to brief darkness or "light sensitized" retinas (Mangel and Dowling 1985; Tornqvist, Yang and Dowling 1988). These studies concluded that dopamine was released after prolonged darkness. However, studies investigating the role of dopamine

in regulating other dopamine-mediated activities in the retina, including retinomotor movements (Dearry and Burnside 1988), formation of horizontal cell spinules (Weiler et al. 1988), changes in the particle density of horizontal cell gap junctions (Baldrige, Ball and Miller 1987), and release of GABA from 'H1' horizontal cells (Yazulla and Kleinschmidt 1982), all suggest that dopamine is released in the light. In addition, Shigematsu and Yamada (1988) have found that the presentation of background illumination to a dark-adapted fish retina reduces horizontal cell receptive-field size, suggesting that dopamine is released in the light. Moreover, Weiler, Kolbinger and Kohler (1989) have recently measured dopamine release directly and found evidence for dopamine release only in the light.

To study the effect of light and dopamine on horizontal cell receptive-field size, we have measured horizontal cell receptive-field sizes in normal retinas before and after background illumination. We have found that there is a reduction in the receptive-field size of fish horizontal cells after the presentation of background illumination to a dark-adapted retina. Furthermore, we have found that the diffusion of lucifer yellow through gap junctions was also reduced when background light was presented to a dark-adapted retina.

To determine if the effect of light on horizontal cell receptive-field size and dye coupling was due to dopamine release we also studied the effect of background illumination

on horizontal cell receptive-field sizes in fish retinas which have had their endogenous source of dopamine removed by lesioning with 6-hydroxydopamine (6-OHDA). If the reduction of horizontal cell receptive-field size by light were due to dopamine release it would be expected that background illumination would not alter horizontal cell receptive-field size or dye diffusion in these lesioned retinas. We found, however, that background illumination reduced horizontal cell receptive-field size and dye diffusion even in 6-OHDA-lesioned retinas. Furthermore, the D₁ dopamine receptor antagonist SCH-23390 failed to block the effect of light on horizontal cell receptive-field size. These findings suggest that dopamine release may not be the only retinal mechanism capable of regulating horizontal cell receptive-field size.

4.0 Methods

4.1 Animals and Tissue Preparation

Adult goldfish (Carassius auratus), 15-20 cm in length, were maintained on a 12 hr light-12 hr dark lighting cycle. Fish were placed in darkness for at least 2 hrs, near the middle of the light phase of the cycle, prior to the beginning of an experiment. Fish were killed by decapitation, enucleated, eyes hemisected and the resulting eyecup inverted over filter paper under dim red light. The sclera, choroid and pigment epithelium were then gently pulled away from the retina and removed by cutting the optic nerve. The retina was then gently lifted off the filter paper by immersion in

Ringer's solution (110 mM NaCl, 2.5 mM KCl, 20 mM NaHCO₃, 1.2 mM MgCl₂, 50 μM CaCl₂, 20 mM glucose, bubbled continuously with 95% O₂, 5% CO₂ maintained at 20°C and at pH 7.2) and transferred to a plastic superfusion dish. A nylon mesh was placed over the retina to hold it in place. The isolated retina preparation was then superfused continuously at a rate of 4 ml/min with Ringer's solution.

4.2 Stimuli

Stimuli were presented to the scleral side of the retina using a dual beam optical bench under computer control. Illumination was produced by a 250 watt tungsten-halogen bulb. One channel presented stimuli of different sizes and shapes (spots, annuli), wavelengths and intensities. A red (640 nm) spot 1.0 mm in diameter at 4.3×10^{12} quanta cm⁻² s⁻¹ was used as stimulus during microelectrode penetration. Stimuli were typically presented for 350 msec every 3 sec. The second channel of the optical bench was used to provide full-field white background illumination.

4.3 Intracellular recording

Glass microelectrodes were pulled on an Alexander-Nastuk puller, filled with 2.5M KCl, and had resistances ranging from 40-100 MΩ. The microelectrodes were advanced into the retina using a piezoelectric microdrive (Burleigh Inchworm) under computer control. Horizontal cell responses were identified and categorized according to their characteristic waveform, large receptive-field size, pattern of responses to chromatic

stimuli, depth of the recording microelectrode, and, when lucifer yellow filled microelectrodes were used, from their morphology after lucifer yellow injection. The most frequently studied cell was the H2 horizontal cell (62%) but a few H1 horizontal cells (20%) and H3 horizontal cells (18%) were also recorded. Horizontal cell responses were amplified (Dagan 8700 Cell Explorer), viewed on an oscilloscope and recorded on magnetic tape for later playback or plotting.

4.4 Measurement of horizontal cell receptive-field size

The receptive-field sizes of horizontal cells were assessed by comparing the responses of spots and annuli of similar illuminated area. Four pairs of spots and annuli were used with diameters as shown in Table 1. White light stimuli with an intensity of 9.8×10^{-7} W/mm² was used for each pair.

If the receptive-field size of horizontal cells is large, spot and annular stimuli of similar illuminated area should evoke similar changes in horizontal cell potential if the annulus ring is not presented too distant from the centre of the horizontal cell receptive field. Since horizontal cell receptive-field size is a function of the coupling between horizontal cells, the response of a horizontal cell to a peripheral stimulus (annulus) of a similar area as a central stimulus (spot) should evoke responses of similar magnitude if horizontal cell coupling is strong. The peripheral stimuli evoke a local change in potential at distant horizontal cells and the resulting current passes within the horizontal cell

Table 1-- Diameters and areas of spot and annulus pairs used to assess horizontal cell receptive-field size.

Pair No.	Spot diameter	Spot Area	Annulus inner diameter	Annulus outer diameter	Annulus area
1	1.25 mm	1.23 mm ²	0.75 mm	1.50 mm	1.33 mm ²
2	2.75 mm	5.94 mm ²	2.50 mm	3.75 mm	6.14 mm ²
3	4.50 mm	15.9 mm ²	2.50 mm	5.25 mm	16.7 mm ²
4	8.00 mm	50.3 mm ²	3.00 mm	8.00 mm	43.2 mm ²

syncytium through gap junctions. However, if horizontal cell coupling is weak and horizontal cell receptive-field size decreases, it would be expected that annular stimuli would evoke a smaller responses to spot stimuli of a similar illuminated area because the current no longer flows as readily from periphery to centre.

4.5 Treatments

After recording responses for each of the four stimulus pairs in two or more cells, a full-field background illumination of 2.5×10^{-7} W/mm² was applied to the retina for 5 min. Horizontal cell responses were recorded for the same stimuli pairs and in the same sequence as were recorded before the application of background illumination. In other experiments, retinas were treated with brief pulses of either 20 μ M dopamine (Sigma) dissolved in Ringer's solution containing 0.1 mM ascorbic acid (Sigma) or 10 μ M SCH-23390 maleate (Schering Canada) dissolved in Ringer's solution. Drug-containing solutions were presented to the retina as superfusate using a valve which allowed switching between normal and drug-containing solutions.

4.6 Dye injection

Microelectrodes were filled with 5% lucifer yellow (Sigma) in 0.1M LiCl₂ and had resistances ranging from 100 to 300 M Ω . horizontal cells were injected with dye by delivering 500 msec 10 nA hyperpolarizing current pulses every 3 secs for 4-5 min. After waiting 20 min for the dye to diffuse, the

retina was fixed in 4% paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.3 for 1 hr and then washed overnight in 0.1M sodium phosphate buffer, 3% sucrose, 0.2 mM CaCl₂, pH 7.3. Retinas were cleared in a 50% glycerol in phosphate buffered saline and viewed under a Zeiss epifluorescence microscope.

Lucifer yellow was injected into horizontal cells from dark-adapted retinas both before and after presentation of full-field background illumination for 4-5 min. The responses of the horizontal cells to spot-annulus pairs of similar illuminated area were always recorded before the injection of lucifer yellow.

4.7 6-hydroxydopamine lesioning

We have previously found a 6-hydroxydopamine lesioning protocol which was determined to be effective by monitoring the uptake of [³H]-dopamine using light microscopic autoradiography (Baldrige, Ball and Miller 1989). Briefly, fish were given a 50 µg intraocular injection of 6-hydroxydopamine (Sigma) on the first and second days of a 14 day time course. The effectiveness of the lesioning was monitored in each population of fish injected with 6-hydroxydopamine in three ways: 1) Uptake of [³H]-dopamine followed by light microscopic autoradiography (Baldrige, Ball and Miller 1989), 2) Tyrosine hydroxylase and serotonin immunohistochemistry, and 3) High pressure liquid chromatography.

4.8 Immunocytochemistry

Hemisected goldfish eyes were fixed in 4% paraformaldehyde in 0.1M phosphate buffer pH 7.3 containing 1% sucrose and 0.2 mM CaCl_2 for 1 hr and then washed for 2 hrs in Rinse Buffer (0.1M sodium phosphate buffer pH 7.3, 3% sucrose, 0.2 mM CaCl_2). Eyes were cryoprotected in 15% sucrose in Rinse Buffer overnight, mounted in OCT compound, 10-15 μm frozen sections cut on a cryostat, sections picked up on subbed glass slides, and dried for 2 hrs at room temperature. Slides were washed in phosphate buffered saline (PBS) to remove OCT compound, 100-200 μl PBS containing 1% normal goat serum (NGS) placed on the sections in a humid atmosphere at room temperature for 10 min. The PBS was replaced with two primary antisera (Anti-tyrosine hydroxylase, Eugene Tech, rabbit, #TE101, 1:500; Anti-serotonin, Eugene Tech, rat, #NT101, 1:500) and incubated overnight. Slides were washed twice for 10 min each in PBS and two secondary antisera placed on the sections (Goat anti-rabbit FITC, Jackson Immunoresearch Labs #111-016-003, 1:100; Goat anti-rat Texas Red, Jackson Immunoresearch Labs #112-076-003, 1:100) for 4 hours. Slides were washed twice for 10 min each and coverslipped in 50% glycerol-PBS containing 0.1% p-phenylenediamine. Subsequent viewing of immuno-labelling of the two antigens in the same section was possible by switching filter sets (Zeiss Standard epifluorescence microscope; FITC: excitation 450 nm - 490 nm,

long pass 520 nm; Texas Red: excitation 510 nm - 560 nm, long pass 590 nm.).

4.9 High pressure liquid chromatography.

Goldfish were dark adapted for 20 min and the retinas isolated in cold Ringer's solution. The vitreous was removed by gentle suction on a 0.45 μ m Millipore filter and weighed. Retinas were homogenized in 200 μ l of 0.2N perchloric acid, immediately frozen on dry ice, and stored at -70°C until analysis. When ready for analysis, samples were thawed, centrifuged, extracted on alumina and applied to the column. An electrochemical detection system was used to measure catecholamines in the samples.

4.10 Effect of the dopamine D₁ antagonist SCH-23390 on dopamine and background illumination.

The ability to block the light- and presumed dopamine-dependent reductions in horizontal cell responses to annular stimuli was tested in unlesioned retinas using the D₁ dopamine receptor antagonist SCH-23390. Retinas were treated for 10 min with Ringer's superfusate containing 10 μ M SCH-23390 maleate. The concentration of SCH-23390 used was the same as that previously described by Yang, Tornqvist and Dowling (1988). This treatment was followed by a 2-4 min pulse of 20 μ M dopamine in Ringer's solution containing 0.1 mM ascorbic acid to determine if the prior treatment with the dopamine antagonist was effective. A background illumination was applied 10 - 15 min later.

5.0 Results

5.1 Horizontal cells in dark-adapted retinas respond similarly to spot and annular stimuli of similar illuminated area.

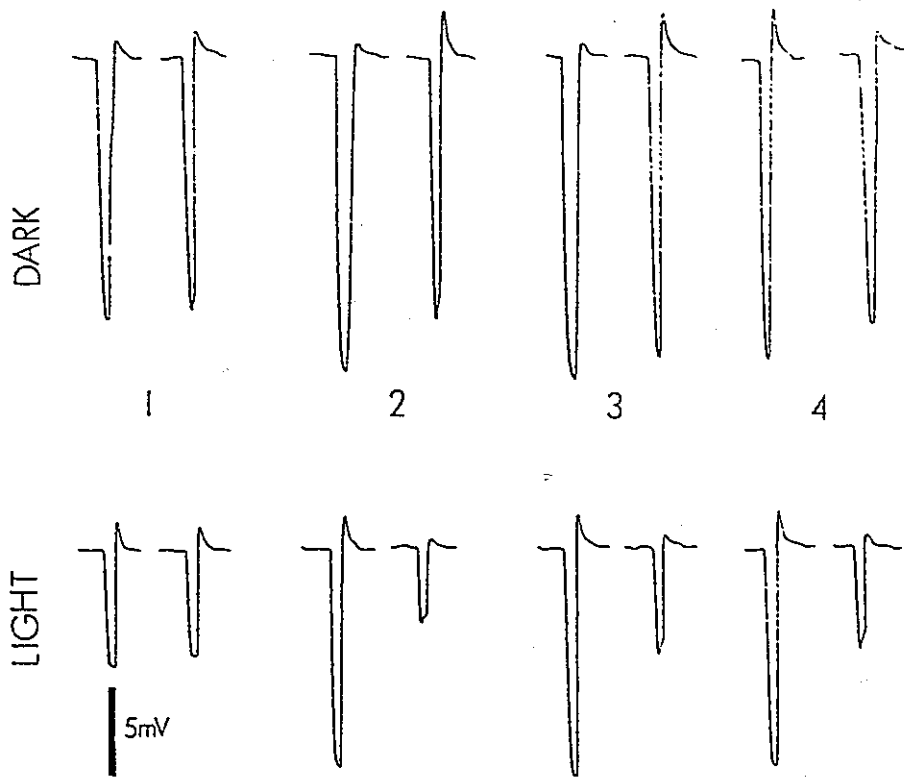
The responses of a horizontal cell to four pairs of spot and annular stimuli are shown in Fig. 1. The dark resting potential of dark-adapted horizontal cells was usually about -25 mV. In dark-adapted retinas, the responses to annular stimuli were nearly as robust as the responses to spot stimuli of a similar illuminated area (Fig. 1, DARK). An index of the strength of annular stimuli responses compared to spot stimuli responses to spot stimuli was determined by calculating the ratio of the annulus response/spot response. The ratio of annulus/spot response was usually better than 0.80 but spot-annulus pair #2 showed a somewhat lower ratios of about 0.70. The similar responses of horizontal cells to spot and annular stimuli of similar illuminated area is indicative of a large horizontal cell receptive-field size.

5.2 Background illumination and dopamine reduce horizontal cell receptive-field size.

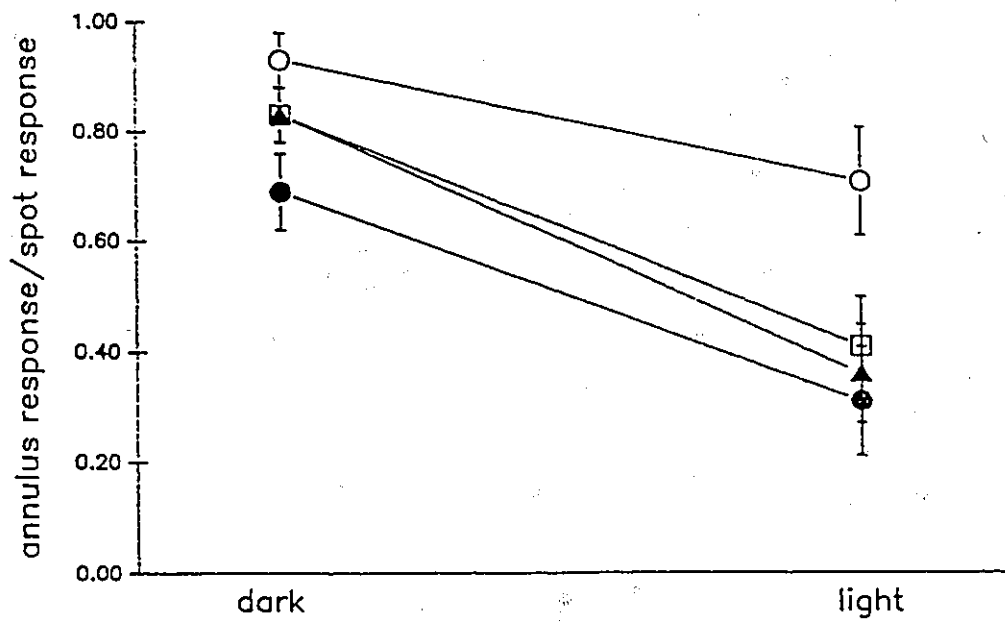
Presentation of background illumination resulted in an initial hyperpolarization of the resting potential to about -45 mV but was usually followed by a gradual depolarization, stabilizing at about -35 mV. After the background illumination was turned off only a small depolarization of about 5 mV was noted, indicating that the dark resting

Figure 1: Responses of a dark-adapted goldfish H2 horizontal cell to white light pairs (1-4) of spot and annulus stimuli of similar illuminated area before (DARK) and after (LIGHT) the retina was exposed to background illumination for 4 min. The record from the light-adapted retina was taken 5 min after the background light was turned off. The dark resting membrane potential of horizontal cells in dark-adapted retinas was about -25 mV before background illumination and about -30 mV after light-adaptation.

Figure 2: Graph showing the mean annulus response/spot response ratios from 14 goldfish horizontal cells before (dark) and after (light) presentation of background illumination to a dark-adapted retina. Open circle annulus/spot pair #1, closed circle pair #2, open square pair #3, closed triangle pair #4. Error bars are 1.7 x standard error of the mean.



①



②

potential after the initial pulse of background light was about -30 mV.

After the presentation of background illumination for 4-5 min, the responses to all types of stimuli were reduced. The responses to the annular stimuli in the largest three pairs showed a marked reduction (Fig. 1, LIGHT). The mean annulus/spot ratios from 14 cone horizontal cells before and after background illumination are shown in Fig. 2. Except for the smallest stimulus pair, the annulus/spot ratio for each of the spot-annulus pairs decreased after presentation of background illumination (Fig. 2). The most common horizontal cell type studied were H2 horizontal cells, however similar results were also observed in H1 and H3 horizontal cells. The decrease in the horizontal cell response to annular stimuli, compared to spot stimuli of similar illuminated area, indicated a reduction in horizontal cell receptive-field size.

The effect of background illumination on dark-adapted retinas was mimicked by dopamine. Fig. 3 shows the results of a similar experiment to that shown in Fig. 2, except that the dark-adapted retina was given a 4 min pulse of 20 μ M dopamine added through the superfusate, rather than presented with background illumination. Treatment with dopamine typically hyperpolarized the dark resting potential of horizontal cells to between -30 mV and -40 mV. Before the application of dopamine the responses of horizontal cells to annular stimuli were robust in comparison to spots of a similar illuminated

area (Fig. 3, DARK). After presentation of dopamine (Fig. 3, DOPAMINE), the responsiveness of the cell decreased. There was also a reduction in the responses to annular stimuli compared to spots of a similar illuminated area. The reduction was most marked in the largest of the spot-annulus pairs.

5.3 Background illumination reduces horizontal cell dye coupling.

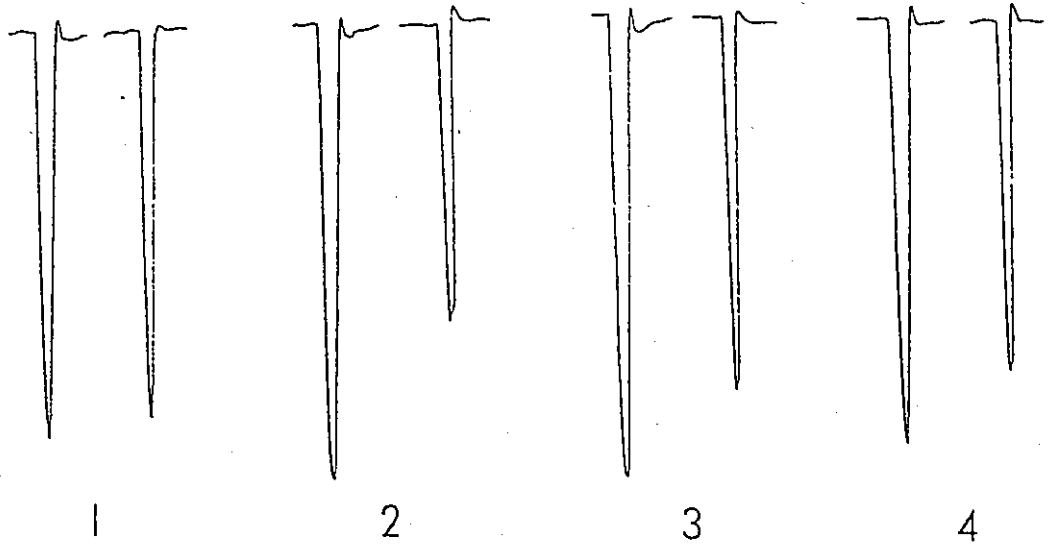
Figure 4 is a fluorescence photomicrograph showing two horizontal cells from the same retina injected with lucifer yellow before (Fig. 4A) or after presentation of background illumination (Fig. 4B). Lucifer yellow spread to numerous adjacent cells before the presentation of background illumination. After the presentation of background illumination there was very little spread of dye to adjacent cells. Reduced diffusion of lucifer yellow was observed in 4 retinas in at least 1 pair of cells (before and after background illumination) in each retina. The responses of these injected cells to spot-annulus pairs, both before and after the presentation of background illumination, were the same as in horizontal cells which had not been injected with dye (Fig. 1).

5.4 Effectiveness of 6-hydroxydopamine lesioning.

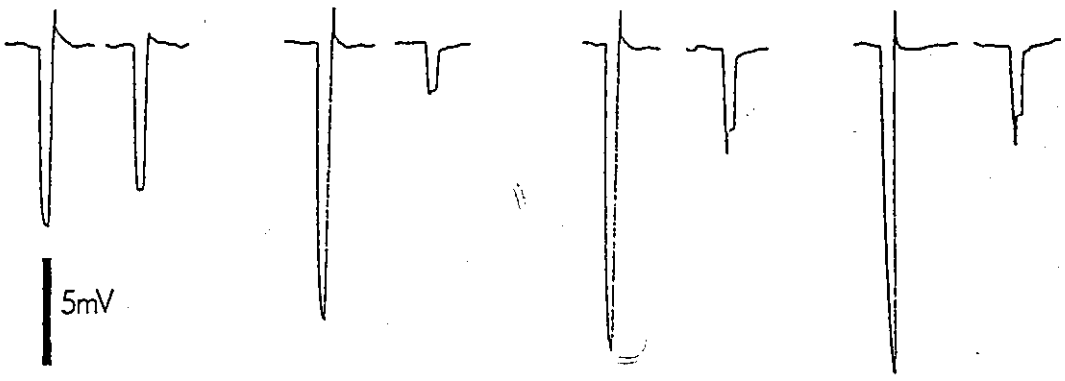
The effectiveness of 100 μ g 6-hydroxydopamine intraocular injections in eliminating retinal dopamine was evaluated in three ways. The lesioning protocol used in the present

Figure 3: Responses of a dark-adapted goldfish H2 horizontal cell to white light pairs (1-4) of spot and annulus stimuli of similar illuminated area before (DARK) or after (DOPAMINE) treatment with a 4 min pulse of 20 μ M dopamine. The dopamine-treated record was taken 10 min after the pulse of dopamine. The dark resting membrane potential of the horizontal cell in the dark-adapted retinas was -25 mV before background illumination and was about -35 mV after dopamine treatment.

DARK



DOPAMINE



3

experiments has previously been shown to be effective in eliminating the uptake of [³H]-dopamine using light microscopic autoradiography (Baldrige, Ball and Miller 1989).

The loss of a high affinity uptake system for [³H]-dopamine suggests that dopaminergic interplexiform cells are damaged by 6-hydroxydopamine lesioning. In control retinas prepared in the current experiments, dense labelling due to [³H]-dopamine accumulation occurred over 'I1' interplexiform cell somata and the neurites of these cells in both the inner and outer plexiform layer after two weeks autoradiographic exposure. In lesioned retinas, no labelling of somata or neurites due to [³H]-dopamine uptake were observed even after 4 weeks autoradiographic exposure.

The loss of tyrosine hydroxylase immunoreactivity in dopaminergic neurons also suggests that interplexiform cells were injured by 6-hydroxydopamine lesioning. The ability to localize serotonin immunoreactivity in the same retinal sections suggests that, of the two amine-containing cells in the fish retina, the lesioning was specific for dopaminergic interplexiform cells. In frozen sections of control retinas, somata which were immunoreactive for both tyrosine hydroxylase (Fig. 5A) and serotonin (Fig. 5B) were observed in the proximal inner nuclear layer. Tyrosine hydroxylase-immunoreactive neurons were absent in retinas lesioned with 6-hydroxydopamine (Fig. 5C) but serotonin-immunoreactive neurons were still present (Fig. 5D).

The lowering of endogenous retinal dopamine levels after 6-hydroxydopamine treatment would suggest that interplexiform cells are injured by the lesioning procedure. High pressure liquid chromatography demonstrated that levels of retinal dopamine were decreased by two orders of magnitude after 6-hydroxydopamine lesioning. Lesioned and unlesioned retinas had comparable weights of $10 \text{ mg} \pm 2 \text{ mg}$. Dopamine levels of $31.2 \text{ ng dopamine/ml homogenate}$ were detected in control retinas (Fig. 6A) and were reduced to 0.46 and $0.37 \text{ ng dopamine/ml homogenate}$ in lesioned retinas (Fig. 6B and 6C).

Lesioning resulted in a slight increase of mean annulus response/spot response ratios in dark-adapted retinas (Fig. 8, dark) compared to unlesioned dark-adapted retinas (Fig. 2, dark).

5.5 Background illumination reduces horizontal cell receptive-field size in 6-hydroxydopamine lesioned retinas.

It was hypothesized that the effect of background illumination on horizontal cell receptive-field size was due to dopamine. To test this hypothesis experiments using paired spot-annulus stimuli before and after application of background illumination were repeated on retinas in which the endogenous source of dopamine had been removed by 6-hydroxydopamine lesioning. Before application of background illumination, horizontal cell responses to annuli were nearly as robust as to spots of a similar illuminated area in each of

Figure 4: Fluorescence photomicrograph showing H2 horizontal cells from unlesioned retinas injected with lucifer yellow before (4A) and a H1 horizontal cell after presentation of background illumination (4B). Scale bar = 25 μm .

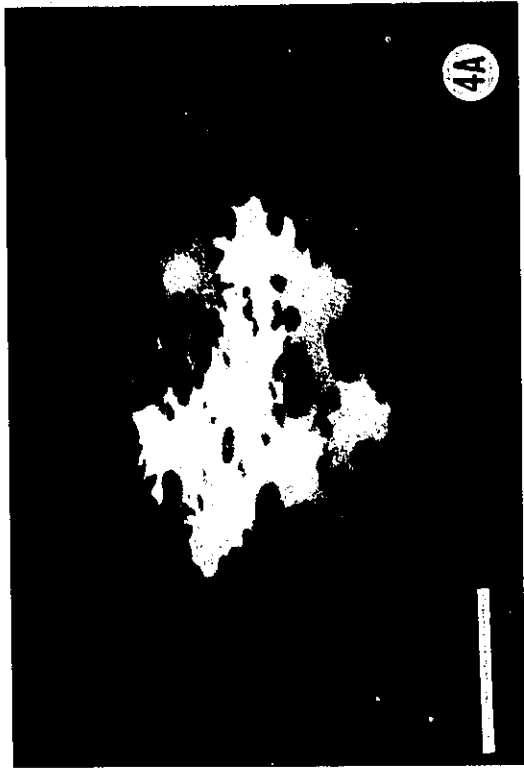


Figure 5: Fluorescence photomicrograph showing double-label immunohistochemical staining for tyrosine hydroxylase (5A, 5C; TH) and serotonin (5B, 5D; 5-HT) in the same section before (5A, 5B) and after (5C, 5D) lesioning with 6-hydroxydopamine. Large arrows indicate immunoreactive somata.

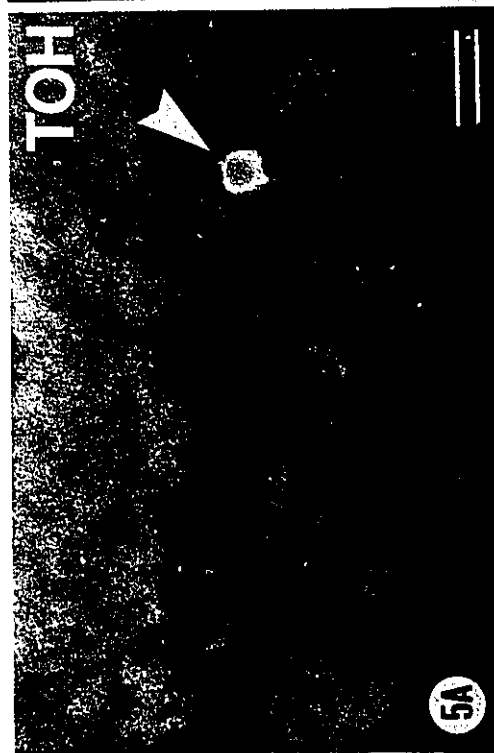
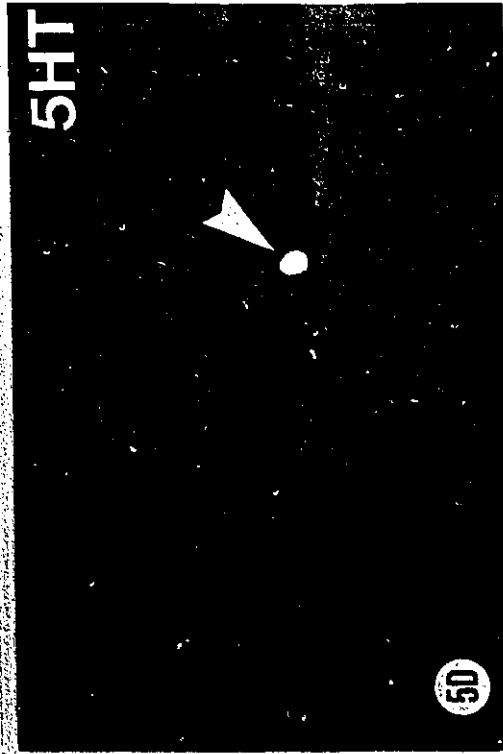
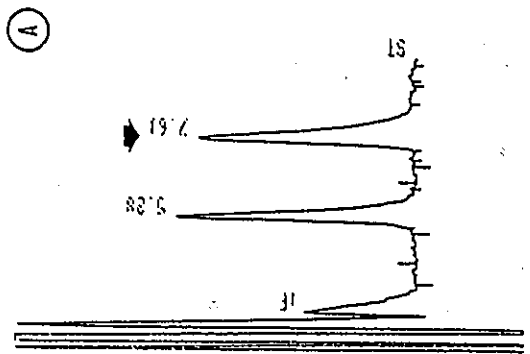
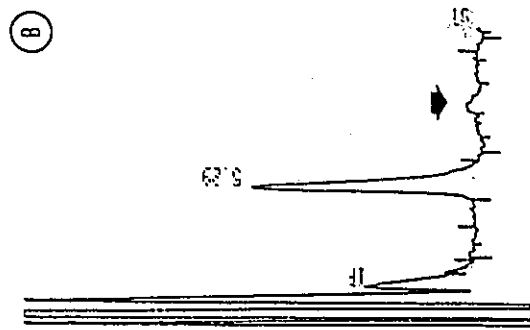
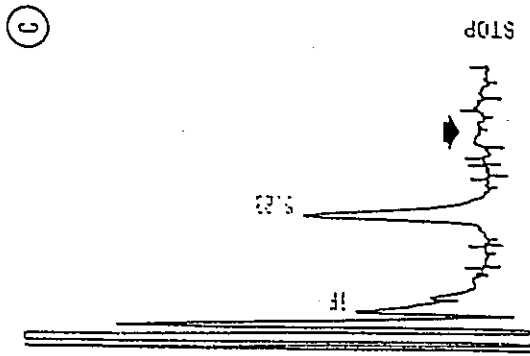


Figure 6: High Performance Liquid Chromatograms of retinal samples measured using electrochemical detection. For the control retinas, 5 μ l of alumina-extracted homogenate was added to the column and the peak represents 31.2 ng dopamine/ml homogenate (6A). For the 6-hydroxydopamine lesioned retinas, 20 μ l of extracted homogenate was added to the column and the peak represents dopamine levels of about 0.40 ng/ml homogenate (6B, 6C). Arrows indicate the normal elution points for dopamine. The other major peak is an internal standard.



the four spot-annulus pairs used (Fig. 7, DARK; Fig. 8, dark). However, after presentation of background illumination the responsiveness of horizontal cells in lesioned retinas to annuli decreased (Fig. 7, LIGHT; Fig. 8, light). As in unlesioned retinas, decreases in horizontal cell responses to annular stimuli were most marked in the largest three of the spot-annulus pairs.

5.6 Background illumination reduces horizontal cell dye coupling in 6-hydroxydopamine lesioned retinas.

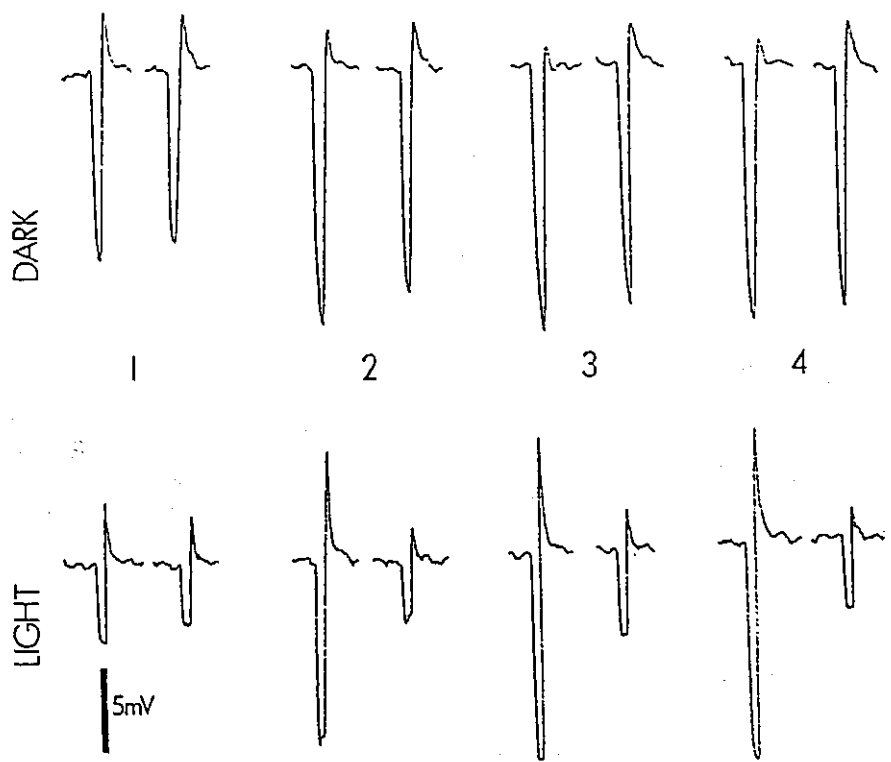
Fig. 9 is a fluorescence photomicrograph showing two horizontal cells from the same 6-hydroxydopamine-lesioned retina injected with lucifer yellow before (Fig. 9A) and after presentation of background illumination (Fig. 9B). As in unlesioned retinas, lucifer yellow spread to numerous adjacent cells before the presentation of background illumination and dye spread was restricted after the presentation of background illumination. These results were observed in 2 lesioned retinas with at least 1 pair of cells (before and after background illumination) in each retina.

5.7 SCH-23390 blocks the effect of dopamine but not light on horizontal cell receptive-field size in unlesioned retinas.

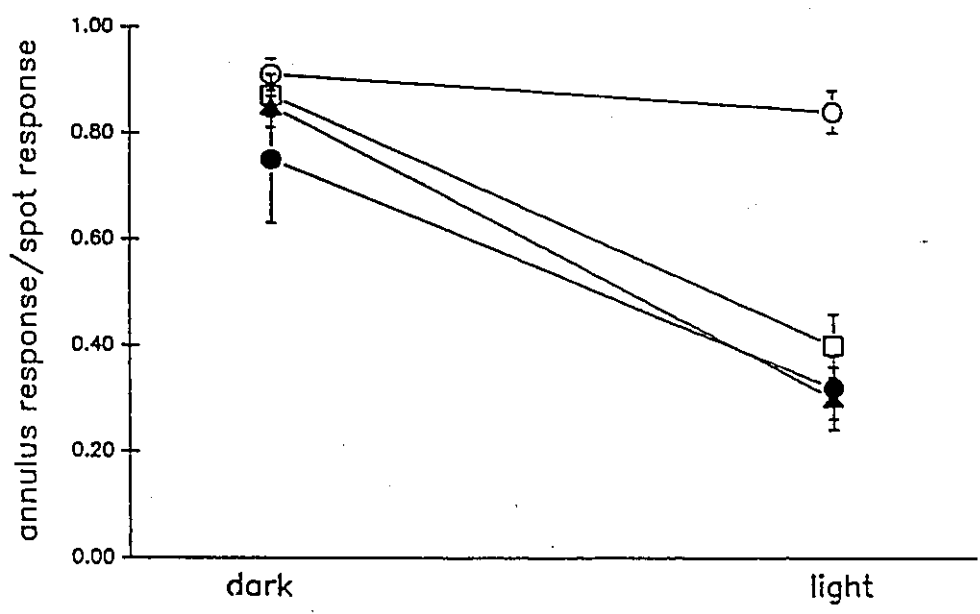
The effect of the D_1 dopamine receptor antagonist SCH-23390 on light- and dopamine-dependent reductions in horizontal cell responses to annular stimuli was also tested. In the absence of SCH-23390, 20 μ M dopamine decreased horizontal cell responsiveness to annular stimuli as described

Figure 7: Responses of a dark-adapted goldfish H2 horizontal cell to white light pairs (1-4) of spot and annulus stimuli of similar illuminated area before (DARK) and after (LIGHT) the retina was exposed to background illumination for 4 min in a dark-adapted 6-hydroxydopamine lesioned retina. The record from the light-adapted retina was taken 5 min after the background light was turned off. The dark resting membrane potential of horizontal cells in dark-adapted retinas was about -25 mV before background illumination and about -30 mV after light-adaptation.

Figure 8: Graph showing the mean annulus response/spot response ratios from 7 horizontal cells before (dark) and after (light) presentation of background illumination to a 6-hydroxydopamine lesioned dark-adapted retina. Open circle annulus/spot pair #1, closed circle pair #2, open square pair #3, closed triangle pair #4. Error bars are 1.7 x standard error of the mean.



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previously (Fig. 3). SCH-23390 blocked the effect of dopamine (Fig. 10, DOPAMINE) but when background illumination was applied the horizontal cell responses to annular stimuli decreased (Fig. 10, LIGHT).

Figure 9: Fluorescence photomicrograph showing H3 horizontal cells from 6-hydroxydopamine lesioned retinas injected with lucifer yellow before (9A) and a H2 horizontal cell after (9B) presentation of background illumination. Scale bar = 25 μ m

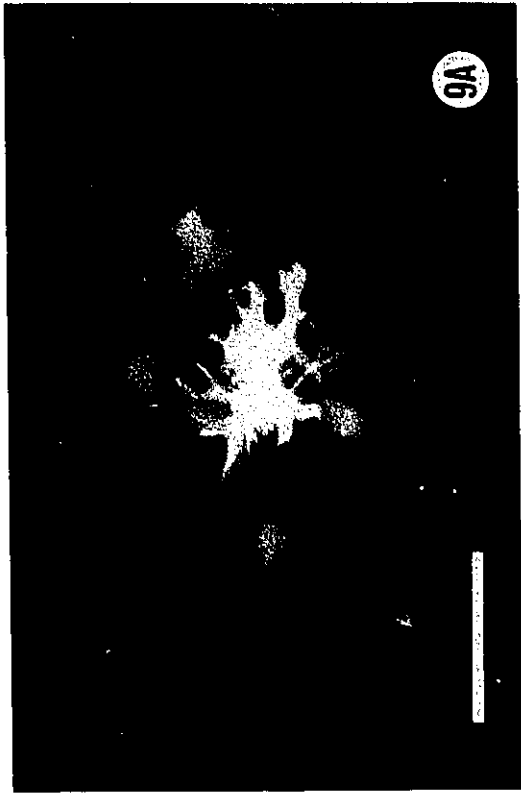
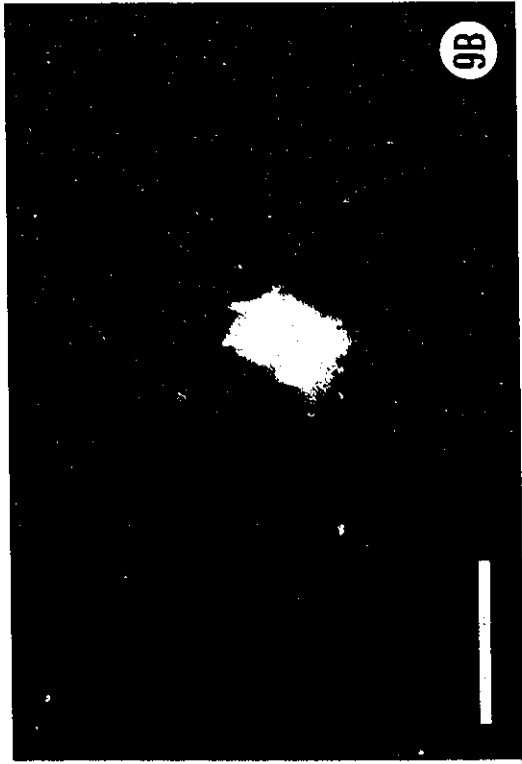
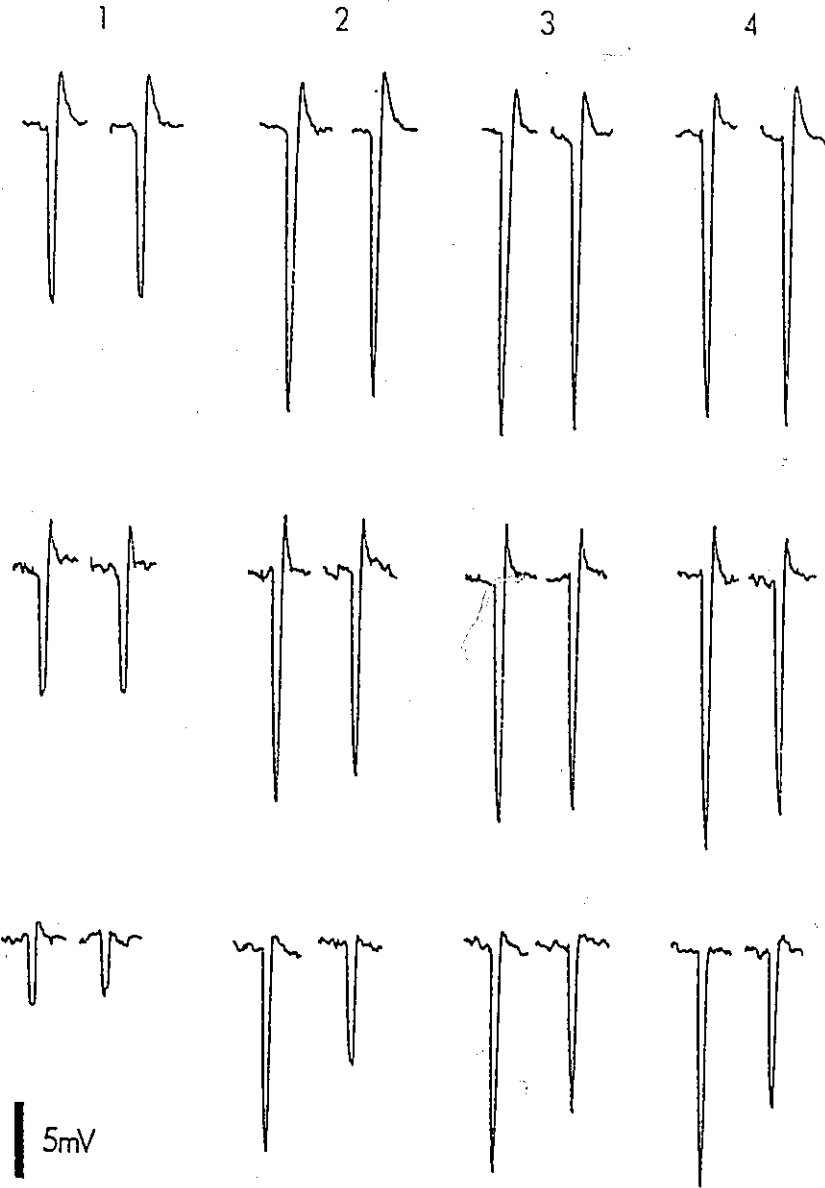


Figure 10: Responses of a H2 horizontal cell to white light pairs (1-4) of spot and annulus stimuli of similar illuminated area in a dark-adapted retinas treated with a 10 min pulse of 10 μ M SCH-23390 (SCH23390), followed by a 2 min pulse of 20 μ M dopamine (DOPAMINE) and finally a 4 min pulse of background illumination (LIGHT).

SCH 23390

DOPAMINE

LIGHT



6.0 Discussion

These experiments demonstrate that the receptive-field size of horizontal cells decreases after the presentation of background illumination to a dark-adapted retina. Similar results have been reported previously in carp retina (Shigematsu and Yamada 1988) and more recently in the mudpuppy retina (Dong and McReynolds 1990). In addition, we have shown that horizontal cell dye coupling was also reduced after background illumination, suggesting that the light-dependent changes in horizontal cell receptive-field size could, at least in part, be attributed to an increase in horizontal cell gap junction resistance. These changes were observed in both normal retinas and retinas lesioned by 6-hydroxydopamine, suggesting that horizontal cell receptive-field size may be regulated by a mechanism other than a dopaminergic system.

It is unlikely that the failure to demonstrate a difference in receptive-field size between lesioned and unlesioned retinas could be explained by an incomplete removal of interplexiform cells or an insufficient reduction in retinal dopamine levels. We assessed the effectiveness of 6-hydroxydopamine lesioning using three different approaches: uptake of [³H]-dopamine, tyrosine hydroxylase immunohistochemistry and high pressure liquid chromatography (HPLC). All three techniques demonstrated that the lesioning protocol eliminated important dopaminergic interplexiform cell markers, and substantially reduced dopamine levels. As a

direct measure of retinal dopamine levels, HPLC revealed a two order of magnitude reduction in retinal dopamine. Furthermore, physiological differences between unlesioned and lesioned retinas were observed, confirming that the lesioning had a demonstrable effect on retinal function. Our results revealed that the receptive-field sizes of horizontal cells in dark-adapted lesioned retinas were increased compared to unlesioned retinas. Similar differences between lesioned and unlesioned retinas have been reported by Teranishi, Negishi and Kato (1984).

A number of studies (see Zigmond et al. 1990 for review) have suggested that lesioning of central dopaminergic neurons with 6-hydroxydopamine may not be sufficient to produce functional loss. This may be due to an increase in dopamine production by surviving neurons, up-regulation of dopamine post-synaptic receptors, or both. Such studies suggest that dopamine levels are maintained by a remaining 10-20% of cells. However, in our lesioned retinas tyrosine hydroxylase-immunoreactive and [³H]-dopamine-accumulating cell somata or neurites were never observed in any retinal sections, suggesting that all interplexiform cells were affected. Furthermore, our HPLC data suggest that the lesioning protocol reduced retinal dopamine levels to near the limit of HPLC detection, two orders of magnitude lower than unlesioned retinas. The possibility remains that dopamine receptors are up-regulated in the 6-hydroxydopamine lesioned fish retina

resulting in increased sensitivity to very low levels of endogenous dopamine.

The changes observed in horizontal cell receptive-field size caused by background illumination may depend on the adaptation state of the retina prior to the application of the background illumination. The observation that background illumination reduces horizontal cell receptive-field size is not necessarily inconsistent with the reports that horizontal cell receptive-field size decreases after prolonged dark-adaptation and increases after presentation of background illumination to prolonged dark-adapted retinas (Mangel and Dowling 1985; Tornqvist, Yang and Dowling 1988). Because prolonged dark-adapted retinas have reduced receptive-field sizes and our dark-adapted retinas had horizontal cell receptive-field sizes which were reduced only after background illumination, the dark-adapted retinas used in our experiments could have been "light-sensitized" (Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988) by brief exposure to light during dissection or initial recording. Such a "light-sensitized" retina would not possess horizontal cells with reduced sensitivity and receptive-field size as seen in prolonged dark-adapted or dopamine-treated retinas. Therefore, the responsiveness and receptive-field size that we and others (Shigematsu and Yamada 1988) describe for dark-adapted retinas may actually correspond to "light-sensitized" dark-adapted retinas. This would suggest that there are two

conditions where the receptive-field size of horizontal cells is reduced, prolonged dark-adapted and light-adapted, and one condition where receptive-field size is increased, "light-sensitized" dark-adapted.

Direct measurements of dopamine release in the fish retina suggest that light stimulates dopamine release (Weiler et al. 1988). We therefore expected that when dopaminergic interplexiform cells were destroyed by 6-hydroxydopamine lesioning, the effect of background illumination on horizontal cell receptive-field size would be diminished. However, the effect of background illumination on horizontal cell receptive-field size in 6-hydroxydopamine lesioned retinas was nearly identical to that observed in unlesioned retinas. This finding suggests that dopamine may not be the only mechanism which governs horizontal cell receptive-field size. Furthermore, if the effect of background illumination on goldfish horizontal cell receptive-field size were solely dopamine-mediated it should be possible to block the effect in unlesioned retinas with appropriate antagonists. Treatment of retinas with the dopamine D₁ receptor antagonists SCH-23390 at 10 μ M did not block the effect of background illumination on horizontal cell receptive-field size. This result contrasts with the results of similar experiments in the mudpuppy retina, in which 15 μ M SCH-23390 was shown to be an effective blocker of light-mediated reductions in horizontal cell receptive-field size (Dong and McReynolds 1990). We do not

believe that the difference between our results and those of Dong and McReynolds (1990) are explained by the small difference in SCH-23390 concentration because our treatment was successful in blocking the effect of 20 μ M dopamine. Therefore, there may be a difference between mudpuppy and goldfish in the mechanism regulating horizontal cell receptive-field size.

It is possible that horizontal cell receptive-field size reduction may also be a D₂ dopamine receptor mediated effect, which would not be blocked by the D₁ antagonist SCH-23390 used in our studies. In Xenopus retina, only a combination of both D₁ and D₂ receptor antagonists were capable of blocking the dopamine-mediated reduction of horizontal cell receptive-field size (Witkovsky, Stone and Besharse 1988). However, in turtle, D₂ antagonists did not block the effect of dopamine (Piccolino and Demontis 1988). Furthermore, our studies do not suggest that a D₂ receptor mediated mechanism could account for the reduction in horizontal cell receptive-field size by background illumination in the presence of SCH-23390. Before the presentation of background illumination to SCH-23390-treated retinas, a pulse of 20 μ M dopamine was given to determine if the antagonist treatment was effective. If a D₂ receptor were involved it would be expected that dopamine would have had an effect, even in the presence of SCH-23390, unless the presentation of background light induced a much

greater release of dopamine at the level of the horizontal cells than accomplished by superfusion with 20 μ M dopamine.

These results do not exclude dopamine release in the light (Weiler et al. 1988). If dopamine is released in the light, however, our results suggest that a dopaminergic system is not the only mechanism by which horizontal cell receptive-field size is reduced in the light. If dopamine played a major role in the light-dependent reduction of horizontal cell receptive-field size it would have been expected that at least some difference in the effect of light between unlesioned and lesioned retinas would have been observed.

In addition to our findings, there is other evidence supporting the possibility of a non-dopaminergic modulation of horizontal cell coupling. DeVries and Schwartz (1989) have reported that the conductance of gap junctions in isolated catfish horizontal cells could be reduced by decreased intracellular pH or cyclic GMP and that levels of these intracellular messengers were unaffected by dopamine. It is therefore possible that a substance, other than dopamine, is released in the fish retina and mediates horizontal cell receptive-field size by changing gap junctional conductance through either cyclic GMP or pH.

An additional mechanism for regulating horizontal cell receptive-field size has recently been proposed by Kamermans, van Dijk and Spekrijse (1990). They suggest that GABA, released in the dark (Marc et al. 1978), acts on horizontal

cell GABA_A receptors modulating a horizontal cell Cl⁻ conductance. The resulting increase in membrane permeability reduces the resistance of the horizontal cell membrane and, therefore, horizontal cell receptive-field size. However, this model was proposed as an alternate explanation for the previously observed reduction of horizontal cell receptive-field size in prolonged dark-adapted retinas (Mangel and Dowling 1985). Because GABA is probably not released from H1 horizontal cells in the light, this model may not account for the reduction in horizontal cell receptive-field size we observed in light-adapted retinas.

Presentation of background illumination to the retina initially hyperpolarized horizontal cells and the membrane potential gradually shifted back to about -30 mV. This effect of background illumination is similar to that reported for horizontal cells in the isolated turtle retina (Normann and Perlman 1990). The reduced responsiveness of horizontal cells following application of background illumination is due, at least in part, to decreased quantal catch after cone photopigment bleaching. Bleaching, however, could not account for the reduced receptive-field size of horizontal cells caused by background illumination. The hyperpolarization of the membrane potential by background light might cause changes in horizontal cell coupling if coupling was voltage-dependent. However, voltage-clamp studies on isolated fish horizontal cells have demonstrated that horizontal cell coupling is not

voltage-dependent (Lasater and Dowling 1985). Therefore, the reduction in horizontal cell receptive-field size we observed after the application of background illumination is not likely due to a voltage-dependent mechanism.

We have previously studied the effect of light-adaptation and dopamine on the freeze-fracture appearance of gap junctions in normal and 6-hydroxydopamine-lesioned retinas (Baldrige, Ball and Miller 1989). It was found that lesioning retinas with 6-hydroxydopamine mimicked the effect of dark-adaptation by elevating gap junction particle density compared to dopamine-treated retinas. Furthermore, light did not reduce gap junction particle density in the 6-hydroxydopamine lesioned retinas, leading us to speculate that dopamine, released in the light, uncouples horizontal cells. The current study, however, demonstrates that horizontal cell receptive-field size and dye-coupling in unlesioned and 6-hydroxydopamine lesioned retinas is reduced after presentation of background illumination. Recently, it has been suggested that changes in horizontal cell gap junction particle density may reflect changes in gap junction renewal and turnover (Vaughan and Lasater 1990) rather than reflect the state of gap junction resistance under different lighting conditions. Therefore, the observed changes in horizontal cell gap junction particle density may represent a light- and dopamine-dependent regulation of gap junction renewal.

In conclusion, we have shown that the action of dopamine may not be the only way horizontal cell receptive-field size can be altered. Specifically, dopamine does not appear to be the major regulator of the light-dependent reduction of horizontal cell receptive-field size. It is possible that some other substance is released in the retina during light-adaptation which mediates this effect. The nature of the substance is unknown but could act by modulating levels of intracellular cGMP or pH thereby reducing horizontal cell coupling through increasing gap junction resistance.

7.0 Acknowledgements

The authors wish to thank Reto Weiler and his co-workers at the Department of Neurobiology, University of Oldenburg for help in horizontal cell intracellular recording. We also thank Dr. John Rowe for developing the electrophysiology system. The HPLC analysis was performed by David Basford, Clinical Chemistry, Department of Pathology, McMaster University. The SCH-23390 was a generous gift from Schering Canada. This work was supported by grants from the Medical Research Council of Canada (to A. Ball) and by a NATO Grant for International Collaboration in Research (to R. Weiler and A. Ball).

III. The effect of background illumination on horizontal cells in the goldfish retina

1.0 Preface and significance to thesis

Part of this work was presented at the 1993 Association for Research in Vision and Ophthalmology meeting (Baldrige and Ball 1993). This chapter represents a draft of a manuscript that will be submitted to the Journal of General Physiology.

The evidence of Mangel and Dowling (1985), Yang, Tornqvist and Dowling (1988a, 1988b) and Tornqvist, Yang and Dowling (1988) suggest that the receptive-field size and responsiveness of horizontal cells, depressed by prolonged darkness, are increased by application of background illumination. After background illumination these retinas were considered to be "light-sensitized" (Yang, Tornqvist and Dowling 1988a). Other studies have demonstrated that application of background illumination to light-sensitized retinas (light-adaptation) reduces horizontal cell receptive-field size (Shigematsu and Yamada 1988; Baldrige and Ball, 1991; Umino, Lee and Dowling, 1991). This paper investigates the relationship between dark-adaptation, light-sensitization and light-adaptation. The paradox, that background illumination can either increase (light-sensitize) or decrease (light-adaptation) horizontal cell responsiveness and

receptive-field size, is resolved in this work by demonstrating that horizontal cells from dark-adapted retinas are first light-sensitized by background illumination and then can be subsequently light-adapted after further exposure to background illumination.

Another source of confusion in the literature pertains to the circumstances under which light-adaptation changes horizontal cell receptive-field size. Some studies report a reduction of horizontal cell receptive-field size only in the presence of background illumination (Umino, Lee and Dowling 1991; Weiler and Akopian 1992). Others reported reduced horizontal cell receptive-field size after background illumination (Baldrige and Ball 1991; Dong and McReynolds 1991) but did not assess receptive-field size during background illumination. This paper examines this difference in approach by showing that the effect of background illumination can reduce horizontal cell receptive-field size both 1) in the presence of background illumination and 2) after the background illumination is turned off.

The reduction of horizontal cell receptive-field size during background illumination was indicated by a relative decrease in the amplitude of response to full-field stimuli compared to slit stimuli. This suggests that the observed changes of receptive-field size were due to a decrease in horizontal cell membrane resistance rather than an increase of gap junction coupling resistance. However, because background

illumination also desensitized horizontal cell responsiveness, it is possible that any increase of gap junction resistance, which would have been indicated by an increase in the response to slit stimuli, was masked.




The reduction of horizontal cell receptive-field size which persisted after background illumination was turned off was detected by a decrease in the amplitude of response to annulus stimuli relative to the amplitude of response to spot stimuli. This change was observed prior to a persistent decrease in the response to full-field stimuli. After additional background illumination, a relative decrease in the amplitude of response to full-field stimuli compared to slit stimuli was also observed. This may indicate that annuli stimuli detect a change in horizontal cell membrane resistance or gap junction resistance, or both, induced by background illumination before such changes are revealed by slit or full-field stimuli.

Analysis of the horizontal cell response waveforms showed that background illumination enhanced a transient hyperpolarizing response at light onset that may be indicative of increased horizontal cell to cone feedback. It is possible that such feedback may also play a role in light-dependent changes in horizontal cell responses to full-field stimuli.

2.0 Abstract

Retinal horizontal cells are the primary inhibitory interneuron of the outer retina and contribute to the surround component of the antagonistic centre-surround receptive fields of bipolar cells. The size of horizontal cell receptive fields exceed the size of the cell, a consequence of extensive coupling by gap junctions. In the present study we have investigated the effect of increased ambient or background illumination on the responsiveness and size of horizontal cell receptive fields. Horizontal cell receptive-field size was assessed by comparing the response to slit and full-field stimuli and, in some cases, by comparing the responses to spot and annulus stimuli.

Background illumination of dark-adapted retina resulted in light-sensitized retinas characterized by increased responsiveness of horizontal cells to slit and full-field stimuli. Application of background illumination to light-sensitized retinas resulted in light-adapted retinas with decreased horizontal cell responsiveness and a reduced response to full-field stimuli relative to slit stimuli. The effect of background illumination on light-sensitized retina occurred, at first, only when background illumination was present. Additional exposure to background illumination, however, produced a reduction of horizontal cell responses to full-field stimuli relative to slit stimuli that persisted even after background illumination was turned off.



Interestingly, a persistent reduction in the response to annulus stimuli was noted in horizontal cells subject to background illumination prior to a persistent reduction in the response to full-field stimuli.

The decrease in the horizontal cell response to full-field stimuli relative to slit stimuli suggests that horizontal cell receptive-field size is decreased by background illumination due to a decrease in horizontal cell membrane resistance. Although the change in horizontal cell receptive-field size during background illumination may be due to a mechanism that responds to the hyperpolarization of the horizontal cell under such conditions, the mechanism that results in a persistent change in horizontal cell receptive-field size is unknown.

3.0 Introduction

Horizontal cells are second-order neurons of the vertebrate retina that provide lateral inhibitory input to cones and bipolar cells. This interaction generates the receptive field surround of bipolar cells (Werblin and Dowling 1969; Werblin 1970; Toyoda and Tonosaki 1978; Toyoda and Kujiraoka 1982) and ultimately contributes to the surround of ganglion cells (Naka and Nye 1971; Naka and Witkovsky 1972; Naka 1977; Sakuranga and Naka 1985). In the goldfish three types of cone horizontal cells and one type of rod horizontal cell have been identified (Stell 1967; Stell and Lightfoot 1975; Weiler 1978).

Horizontal cells are extensively coupled (Naka and Rushton 1967) by gap junctions that connect horizontal cells of the same type (Yamada and Ishikawa 1965; Stell 1967; Witkovsky and Dowling 1969; Kaneko and Stuart 1980, 1984). One consequence of electrotonic coupling is that the receptive-field size of horizontal cells is much larger (≥ 2 mm) than the size of their dendritic field (≈ 100 μm).

Horizontal cells receive direct input from dopaminergic interplexiform cells (Ehinger, Falck and Laties 1969; Sarthy and Lam 1979; Marc 1982; Yazulla and Zucker 1988) and dopamine has been shown to reduce horizontal cell responsiveness and receptive-field size (Hedden and Dowling 1978; Negishi and Drujan 1979a, 1979b; Negishi, Teranishi and Kato 1983; Teranishi, Negishi and Kato 1984; Mangel and Dowling 1985).

Because the responsiveness and receptive-field size of fish horizontal cells are also reduced after prolonged dark-adaptation (Mangel and Dowling 1985; Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988a, 1988b), it has been suggested that dopamine, released from interplexiform cells in the dark, was responsible for the observed reduction of horizontal cell activity after prolonged dark-adaptation. Furthermore, Yang, Tornqvist and Dowling (1988a) suggested that horizontal cells from dark-adapted retinas are "light-sensitized" by background illumination resulting in increased responsiveness and receptive-field size believed to be the result of decreased dopamine release.

In apparent contradiction to these reports, studies have also demonstrated that background illumination, rather than darkness, reduces the receptive-field size of horizontal cells (Shigematsu and Yamada 1988). However, Baldrige and Ball (1991) and Umino, Lee and Dowling (1991), who also report light-induced reduction of horizontal cell receptive-field size, demonstrated that this effect of background illumination, unlike darkness, was not mediated by dopamine. It was argued that the retinas exposed to background illumination in these experiments were not dark-adapted but light-sensitized. Therefore, when horizontal cells from dark-adapted retinas are exposed to background illumination the receptive-field size should first increase (light-sensitize)

and then, if subject to continued or additional background illumination, decrease (light-adaptation).

Background illumination also reduces the receptive-field size of mudpuppy (Dong and McReynolds 1991, 1992) and turtle (Weiler and Akopian 1992) horizontal cells. In contrast to fish horizontal cells, however, the light-dependent change in mudpuppy and turtle horizontal cells can be blocked by dopamine-receptor antagonists (Dong and McReynolds 1991, 1991; Weiler and Akopian 1992). The source of dopamine in the retina of mudpuppy and turtle is a population of amacrine cells, rather than interplexiform cells as found in the fish (Adolph, Dowling and Ehinger 1980; Nguyen-Legros et al. 1985b). The different type of dopaminergic neurons in mudpuppy and turtle may, therefore, be related to the apparent difference in the light-dependent changes of horizontal cell activity.

Two effects of background illumination on horizontal cell receptive-field size have been reported. In some studies horizontal cell receptive-field size was reported to decrease only in the presence of background light and did not persist after the background illumination was turned off (Umino, Lee and Dowling 1991; Weiler and Akopian 1992). In other studies, the effect of background illumination on horizontal cell receptive-field size was not determined when the background illumination was on but was found to be decreased after the background illumination was turned off (Baldrige and Ball

1991; Dong and McReynolds 1991). In the latter case complete recovery was noted within minutes after the background illumination was turned off (Dong and McReynolds 1991). Shigematsu and Yamada (1988) reported that horizontal cell receptive-field size was decreased during background illumination and that this effect persisted for several minutes after the background illumination was turned off.

In the current study, horizontal cell responsiveness and receptive-field size were studied in dark-adapted goldfish retinas during and after background illumination.

4.0 Methods

4.1 Animals

Adult goldfish (*Carassius auratus*), 10-12 cm in length, were obtained from a commercial supplier (Tropical Fish Supply, Fort Erie, ON), maintained under a 12 hr:12 hr light:dark cycle in continuous water flow tanks at about 15°C. Prior to study fish were dark-adapted for 1-2 hrs in an opaque aerated tank.

4.2 Preparation of retina

Preparation of the isolated retina was performed under dim red light. Fish were killed by decapitation and pithing. Eyes were enucleated, hemisected, and the eyecup inverted onto filter paper. The sclera, choroid and retinal pigmented epithelium was gently lifted away after sectioning the optic nerve. After moistening the filter paper with Ringer's solution, the retina was transferred to an acrylic superfusion

chamber photoreceptor-side up. A small net held the retina in place while the retina was superfused continuously with Ringer's solution at a rate of 1-2 ml/min. The Ringer's solution consisted of 110 mM NaCl, 2.5 mM KCl, 20 mM NaHCO₃, 0.1 mM MgCl₂, 0.7 mM CaCl₂, 20 mM D-glucose, and was bubbled continuously with 95% O₂, 5% CO₂ which resulted in a pH of 7.2.

4.3 Light stimulus

Light was projected onto the vitreal side of the retina through the quartz bottom of the acrylic superfusion chamber. Light was supplied by a dual-beam optical bench under computer control using a 250 watt tungsten-halogen bulb. Unattenuated light intensity (I_0) was $10^4 \mu\text{W cm}^{-2}$ (7×10^{13} photons $\text{cm}^{-2} \text{sec}^{-1}$ at 500 nm). One channel provided a full-field of white light at a fixed intensity of $-3.7 \log I_0$. The other channel could project a variety of stimuli of different intensity, colour and shape. An additional source of full-field illumination was provided by a 60-watt tungsten bulb that projected a diffuse background illumination on the photoreceptor-side of the retina. All intensities are reported in reference to I_0 of the optical bench.

4.4 Intracellular recording

Borosilicate glass (WPI, Sarasota, FL) microelectrodes prepared using a Brown/Flaming puller (Sutter Instruments, Novato, CA) were filled with 3.0M potassium acetate and had resistances around 60-100 M Ω . Microelectrodes were advanced into the retina using a piezoelectric microdrive (Burleigh

Inchworm, Burleigh Instruments, Fishers, NY) under computer control. Microelectrodes were aligned to the optical axis of the optical bench using an infrared filter (> 800 nm) and an infrared-imaging camera (Electrophysics, Nutley, NJ). Horizontal cells were identified and categorized according to their characteristic waveform, large-receptive-field size, pattern of responses to chromatic stimuli and from the depth of the recording microelectrode. All recordings reported here came from H2 horizontal cells. Horizontal cell responses were amplified (Dagan 8700 Cell Explorer, Dagan Corporation, Minneapolis, MN), digitized and recorded onto a computer hard-disk (Axotape, Axon Instruments, Foster City, CA). Recordings were analyzed and plotted using graphics software (Sigmaplot, Jandel Scientific, San Rafael, CA).

4.5 Assessment of horizontal cell receptive-field size

Because the size of the horizontal cell receptive-field is a function of the resistance of both gap junctions (R_i) connecting horizontal cells and the resistance of the horizontal cell membrane (R_m), horizontal cell receptive-field size will decrease when either R_i increases, R_m decreases or both change (Lamb 1976). Therefore, horizontal cell responses to centred small spot or slit stimuli will increase when horizontal cell gap junction resistance increases because the input resistance of the horizontal cell network increases as R_i increases (Piccolino, Neyton and Gerschenfeld 1984; Teranishi, Negishi and Kato 1984). Horizontal cell responses

to full-field stimuli, however, should not be affected by changes in R_i , because all the horizontal cells in the syncytium will be equipotential (Mangel and Dowling 1987). However, when R_m decreases the response to full-field stimuli will decrease as more current passes across the membrane rather than through gap junctions of connecting horizontal cells (Mangel and Dowling 1987).

Changes in the receptive-field size of horizontal cells were determined by comparing the responses to a white light slit stimulus (250 μm x 7 mm) and a full-field stimulus. A slit was used instead of a small spot because of the ease with which it could be aligned with the electrode. The intensity of the slit was adjusted prior to the application of background illumination to produce a response amplitude similar to that of full-field stimulus at a fixed intensity. Background illumination was then applied and the amplitude of the responses to slit and full-field stimuli determined during and after exposure. Comparison of responses to slit and full-field stimuli before, during and after background illumination were quantified by taking the ratio of slit/full-field response amplitude. Horizontal cell response amplitude was taken to be the maximum hyperpolarizing response obtained at any point during the 350 msec light pulse. Differences between mean values of slit/full-field response ratios were tested for statistical significance using oneway analysis of variance ($\alpha=0.05$). In some experiments the responses of

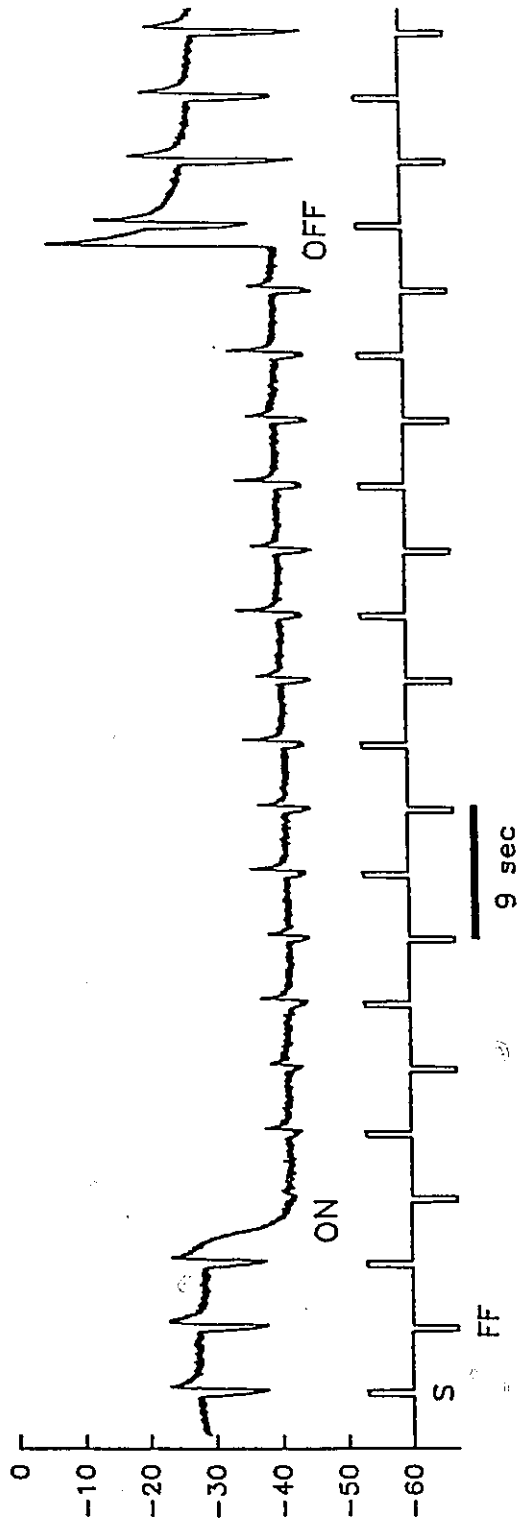
horizontal cells, before and after background illumination, to spot and annulus stimuli were also recorded.

5.0 Results

5.1 Light-sensitization

In retinas dark-adapted for 1-2 hrs, some (3/15) horizontal cells showed increased responsiveness after presentation of background illumination (Figs. 1-3). Prior to the application of background illumination the intensity of slit stimulus was adjusted to produce a response similar in amplitude to the response to full-field stimulus at a fixed intensity (Fig. 1, 3A, S and FF). In the presence of background illumination horizontal cell membrane potential hyperpolarized to a steady level during the duration of background illumination (Fig. 1). During background illumination responsiveness first decreased and then partially recovered. In addition, the response to full-field stimuli increased in comparison to the responses to slit stimuli within 30 sec while the background illumination was on (Fig. 3B). After the background illumination was turned off the horizontal cell depolarized beyond the dark resting membrane potential prior to background illumination. However, the potential quickly returned to the dark resting membrane potential prior to background illumination. After background illumination the response of the horizontal cells to both slit and full-field stimuli were increased compared to before application of background illumination and the response

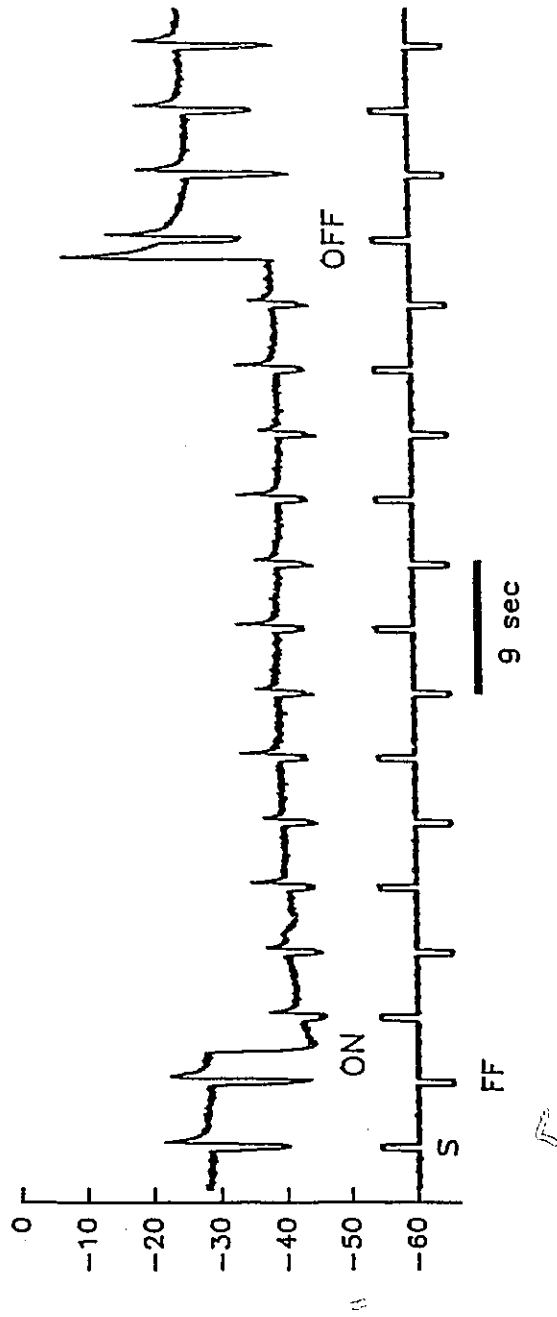
Figure 1 Application of background illumination to a dark-adapted retina results in light-sensitization. Prior to background illumination, the intensity of the slit stimulus (S) was adjusted to produce a response amplitude approximately equal to the response to full-field stimuli (FF). Background illumination was then applied for about 1 minute (ON) which hyperpolarized the cell to a steady level. Horizontal cell responsiveness was initially reduced but gradually increased during background illumination. Immediately before the termination of background illumination (OFF) the response to a full-field stimulus was greater than the response to a slit stimulus. Immediately after the background illumination was terminated (OFF) the horizontal cell responsiveness to both slit and full-field stimulus was increased. In addition, the response to a full-field stimulus increased relative to the response to a slit. The dark resting membrane potential was the same before and after background illumination. Slit and full-field stimulus duration time was 350 msec. Slit intensity was $-2.5 \log I_0$ and full-field intensity was $-3.7 \log I_0$. The intensity of the background light was $-2.5 \log I_0$. The ordinate indicates cell potential in mV.



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Figure 2 The first application of background illumination to a light-sensitized retina may have little effect on horizontal cells. This figure is a continuation of the recording of the cell shown in Fig. 1. When background illumination (ON) of the same intensity as used in Fig. 1 was applied 30 sec after termination of the first exposure to background illumination there was less desensitization and only a slight increase in response amplitude during illumination. During this second application of background illumination the cell showed a slight depolarization. There was no increase in responsiveness after the second background illumination exposure was terminated (OFF) and the relative response of slit (S) and full-field (FF) stimulus remained similar to the relationship prior to background illumination. The dark resting membrane potential after background illumination was slightly depolarized compared to before background illumination. Stimulus time length, intensities and ordinate details are identical to Fig. 1.

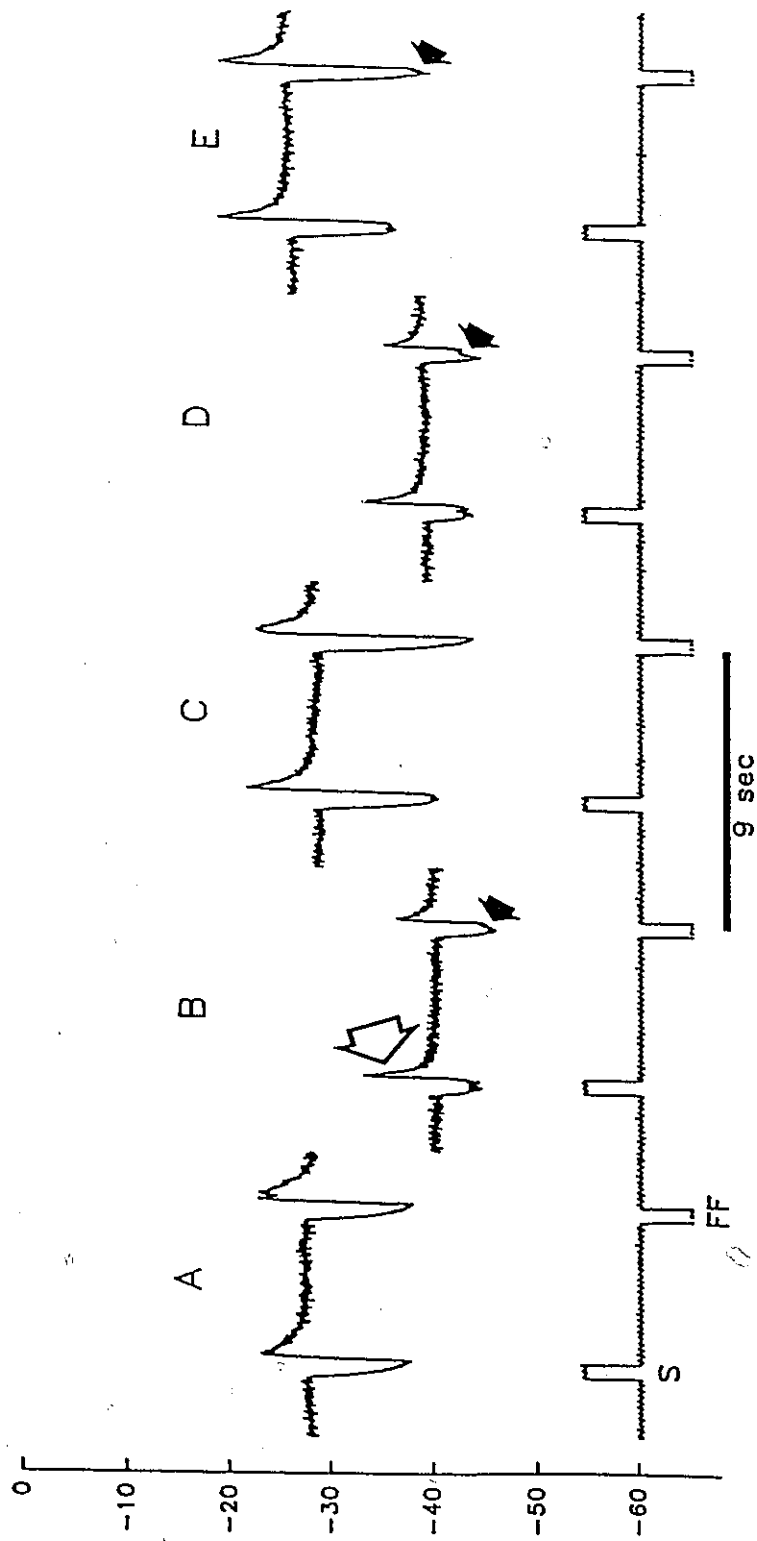


to full-field stimuli was greater than to slit stimuli (Fig. 1, 3C). Additional application of background illumination either slightly decreased or did not alter the response amplitude to slit and full-field stimuli further during and after exposure to background illumination (Figs. 2, 3D, 3E). In addition, the amplitude of the response to full-field stimuli relative to the response to slit stimuli did not change after exposure to a second period of background illumination (Figs. 2, 3E). During this second application of background illumination the membrane potential of the cell hyperpolarized and then gradually depolarized (< 5 mV) (Fig. 2). After background illumination the cell depolarized beyond the dark resting membrane potential prior to illumination but then quickly hyperpolarized back to the same or slightly depolarized (Fig. 2) potential compared to the potential prior to background illumination.

During the first and second exposure of a dark-adapted retina to background illumination, changes in the waveform of the horizontal cell were observed. During (Fig. 3B) and after (Fig. 3C) application of background illumination the responses were faster, but the general shape of its waveform remained unchanged. During the first exposure (Fig. 3B), during the second exposure (Fig. 3D) and after the second exposure (Fig. 3E) to background illumination, a hyperpolarizing transient response developed (Fig. 3, solid arrows) at the onset of light stimuli, particularly to full-field stimuli, followed by

Figure 3 Changes in horizontal cell waveform occur during the progression from a dark-adapted to a light-sensitized retina.

(A) Prior to background illumination the intensity of slit (S) and full-field (FF) stimuli were adjusted to produce similar response amplitudes. (B) During background illumination, just before it was terminated, the response to a full-field stimulus was greater than the response to a slit stimulus. A transient hyperpolarization at stimulus onset appeared in the response to full-field stimulus (filled arrow). A depolarizing off-transient was increased in the response to slit stimulus (open arrow) but not in the response to full-field stimuli. (C) After the first exposure to background illumination the response amplitude to both slit and full-field stimuli were increased. In addition, the response to a full-field stimulus was greater than the response to a slit stimulus. The hyperpolarizing transient observed at light onset in B was not observed after exposure to background illumination even though the kinetics of the response at the onset of both stimuli were faster. (D) During the second application of background illumination the hyperpolarizing transient observed at light onset became more apparent (filled arrow) but the amplitude of responses to slit and full-field stimuli were similar to those seen in B. (E) After the second application of background illumination the response amplitude to slit and full-field stimuli was similar to the response amplitudes before background illumination (C).



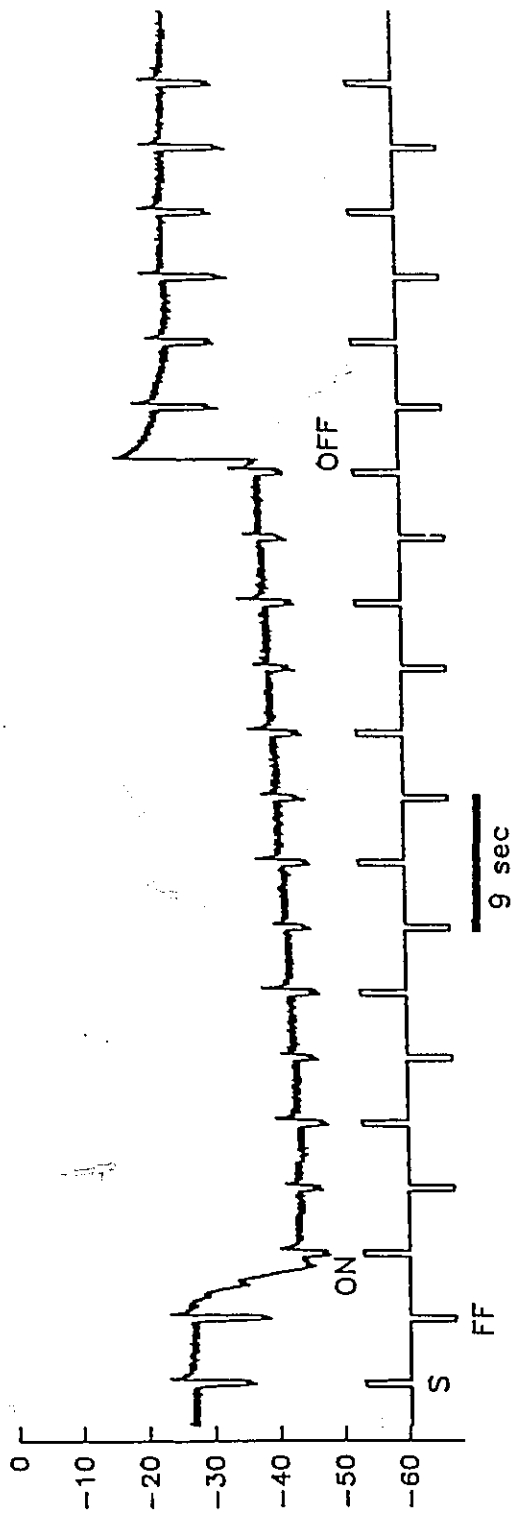
a gradual depolarization. Depolarizing transients at light offset (eg. Fig. 3, open arrow) were frequently observed in horizontal responses to both slit and full-field stimuli and usually increased during and after the presentation of background illumination.

The reported increased responsiveness of horizontal cells from dark-adapted retinas represents light-sensitization of a dark-adapted retina (Yang, Tornqvist and Dowling 1988a). It was also shown that, after light-sensitization, additional exposure to background illumination may not necessarily have an effect on horizontal cells (Fig. 2). However, subsequent application of background illumination ultimately affected horizontal cells and the following experiments demonstrate this phenomenon. Retinas were deemed to have already been light-sensitized if background illumination had no effect or reduced the response to full-field stimuli relative to slit stimuli during or after background illumination.

5.2 Horizontal cell responses in presence of background illumination

Application of background illumination to light-sensitized retinas (Fig. 4, ON) can result in an immediate increase in the response to slit stimuli relative to full-field stimuli. A transient hyperpolarization at light onset was apparent in the response to full-field stimuli and the response kinetics to slit stimuli were increased.

Figure 4 Application of background illumination to a light-sensitized retina reduces the horizontal cell response to full-field stimuli. During application of background illumination (ON) the cell hyperpolarized and then gradually depolarized. During background illumination the response to slit (S) stimuli was greater than the response to full-field (FF) stimuli. After background illumination (OFF) the cell initially depolarized beyond the initial dark resting membrane potential and subsequently hyperpolarized to a potential somewhat ($< 5\text{mV}$) depolarized compared to the initial potential. The responses to both slit and full-field stimuli were both decreased compared to before background illumination. However, the response to full-field stimuli relative to slit stimuli were unchanged after background illumination. Slit and full-field stimulus times were 350 msec. Slit intensity $-2.2 \log I_0$, full-field intensity $-3.7 \log I_0$, background illumination intensity $-2.5 \log I_0$. Ordinate indicates membrane potential in mV.



Prior to presentation of background illumination the response to full-field stimuli was equal or slightly greater than the response to slit stimuli. During the presentation of background illumination the cell hyperpolarized followed by a gradual depolarization. During background illumination the response to full-field stimuli decreased more than the response to slit-stimuli. After the background illumination was turned off (Fig. 4, OFF) the cell initially depolarized beyond the dark resting membrane potential prior to background illumination and subsequently hyperpolarized to a potential somewhat (< 5 mV) depolarized to the initial dark resting membrane potential. After background illumination the response to full-field stimuli was at first reduced, relative to the response to the slit, but the response to full-field stimuli rapidly returned to the level before background illumination (Fig. 4; compare Fig. 5A to 5C).

During and after the application of background illumination the waveform of the horizontal cell responses often showed an increase in the hyperpolarizing transient responses at light onset (Fig. 5, solid arrows) and depolarizing transients at light offset (Fig. 5, open arrow).

5.3 Light-adaptation

In light-sensitized retinas initial exposures to background illumination reduced horizontal cell responses to full-field stimuli relative to slit stimuli only while the background illumination was on and did not persist after it

Figure 5 *Changes in horizontal cell waveform during and after the application of background illumination to a light-sensitized retina.* (A) Prior to background illumination the response to a full-field (FF) stimulus was slightly larger than the response to a slit (S) stimulus. (B) During background illumination the response to the slit was greater than the response to full-field. There was also an increase in the transient hyperpolarization at light stimulus onset (filled arrow) in response to both slit and full-field stimuli. The depolarizing transient at light stimulus offset was decreased in the responses to full-field stimulus (small open arrow) but was not markedly altered in the response to slit stimuli. (C) After background illumination the responses to both slit and full-field stimuli were decreased but the response to a full-field stimulus, relative to the response to a slit stimulus, was similar to that before background illumination (A). The hyperpolarizing transient at light onset was increased in both the responses to slit and full-field stimuli after background illumination (filled arrow) but the depolarizing transient at light off were not changed from the pre-background illumination condition.

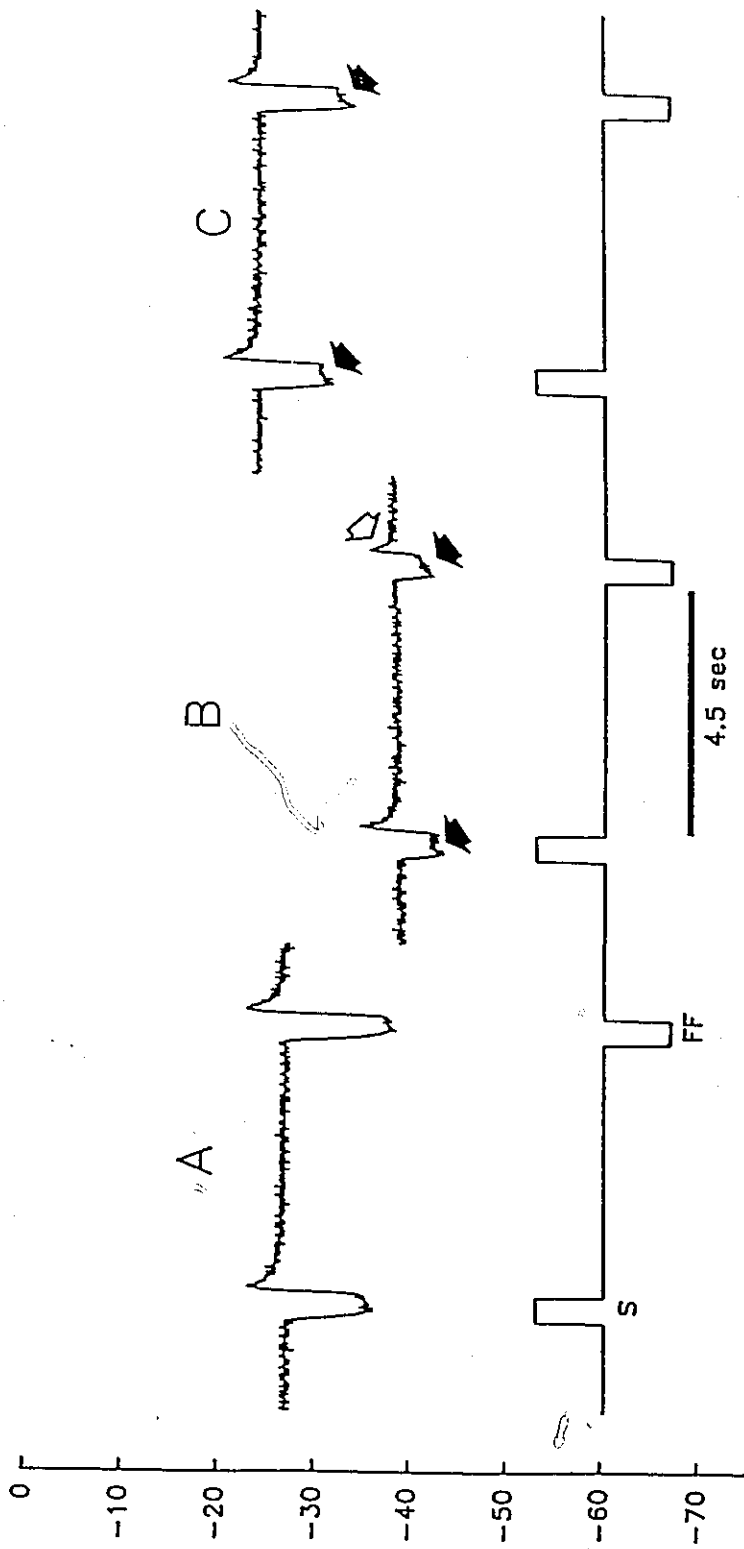
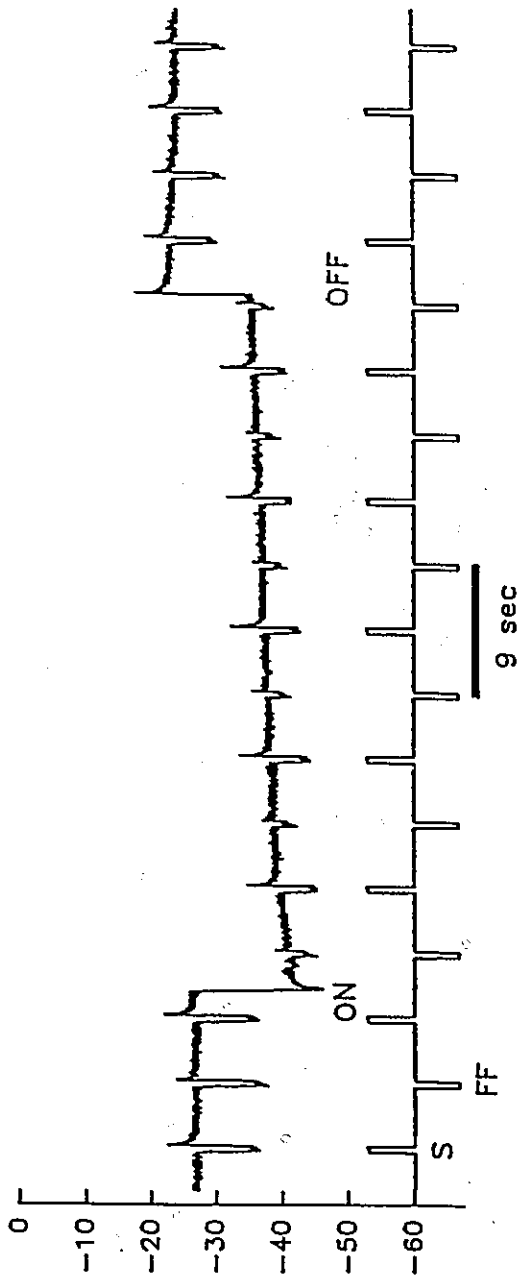


Figure 6 Application of background illumination to a light-sensitized retina. Prior to background illumination the response to full-field (FF) stimulus was slightly greater than the response to slit (S) stimulus. During background illumination (ON) the cell hyperpolarized followed by a gradual depolarization and the response to slit stimulus was greater than the response to full-field stimulus. After background illumination (OFF) the cell depolarized beyond the dark resting membrane potential prior to background illumination and then hyperpolarized to a potential somewhat depolarized (< 5 mV) compared to the dark resting membrane potential prior to background illumination. After background illumination the responses to slit and full-field stimuli were somewhat reduced and nearly identical in amplitude. Slit intensity $-2.3 \log I_0$, full-field intensity $-3.7 \log I_0$, background illumination intensity $-2.5 \log I_0$. Stimulus time length was 350 msec. Ordinate indicates membrane potential in mV.



was turned off (Fig. 4). Eventually, however, an increase in the response to slit stimuli relative to the response to full-field stimuli was observed to persist after background illumination was turned off. The effect was initially small (Fig 6; compare Fig. 7C to 7A) but became more prominent after additional background illumination (Figs. 8, 9C). The observed decrease in horizontal cell response amplitude to full-field stimuli relative to slit stimuli persisted for more than one minute after the background illumination was turned off (Fig. 9D). The persistent change in responses to slit and full-field stimuli only occurred in horizontal cells that also showed decreased responses to full-field stimuli during the presentation of background illumination. The persistent decrease in full-field response after background illumination also always occurred in conjunction with a decrease in horizontal cell responsiveness.

During background illumination horizontal cells hyperpolarized but then gradually depolarized during the period of background illumination (Fig. 6, 8). When the background light was turned off the cell first depolarized beyond the dark resting membrane potential prior to background illumination but then quickly hyperpolarized to a potential somewhat depolarized (< 5 mV) to the dark resting membrane potential prior to background illumination (Fig. 6, 8).

Changes in the waveform of the response to full-field stimuli during and after application of background illumination

Figure 7 Changes in horizontal cell waveform during application of background illumination to a light-sensitized retina. Details of waveform from the horizontal cell in Fig. 6. (A) Before background illumination the response to full-field (FF) stimulus was somewhat greater than the response to slit (S) stimulus. (B) During background illumination the response to slit stimuli was greater than the response to full-field stimulus. The hyperpolarizing transient at light onset in response to full-field stimulus (filled arrow) and the depolarizing transient at light off in response to the slit were increased (large open arrow), and the depolarizing transient at light off to full-field stimulus was decreased (small open arrow). (C) After background illumination the response to both stimuli were decreased but the amplitude of slit and full-field stimuli response were nearly identical. In this cell, the hyperpolarizing transient at light onset (filled arrow), in response to full-field stimulus, was less obvious after background illumination than before or during background illumination. The depolarizing transients at light off were not markedly increased after background illumination in comparison to before (A).

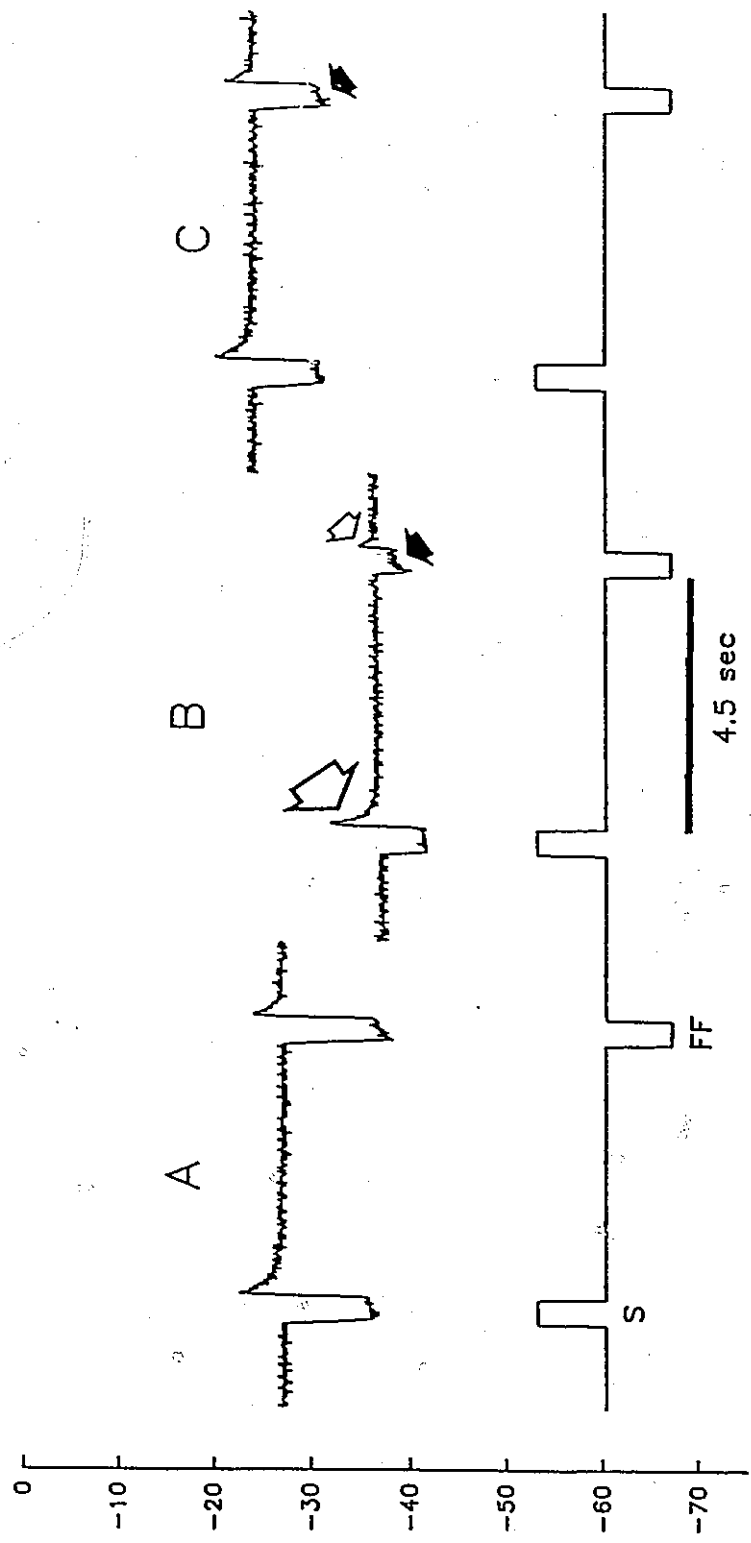
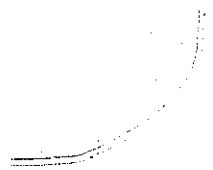
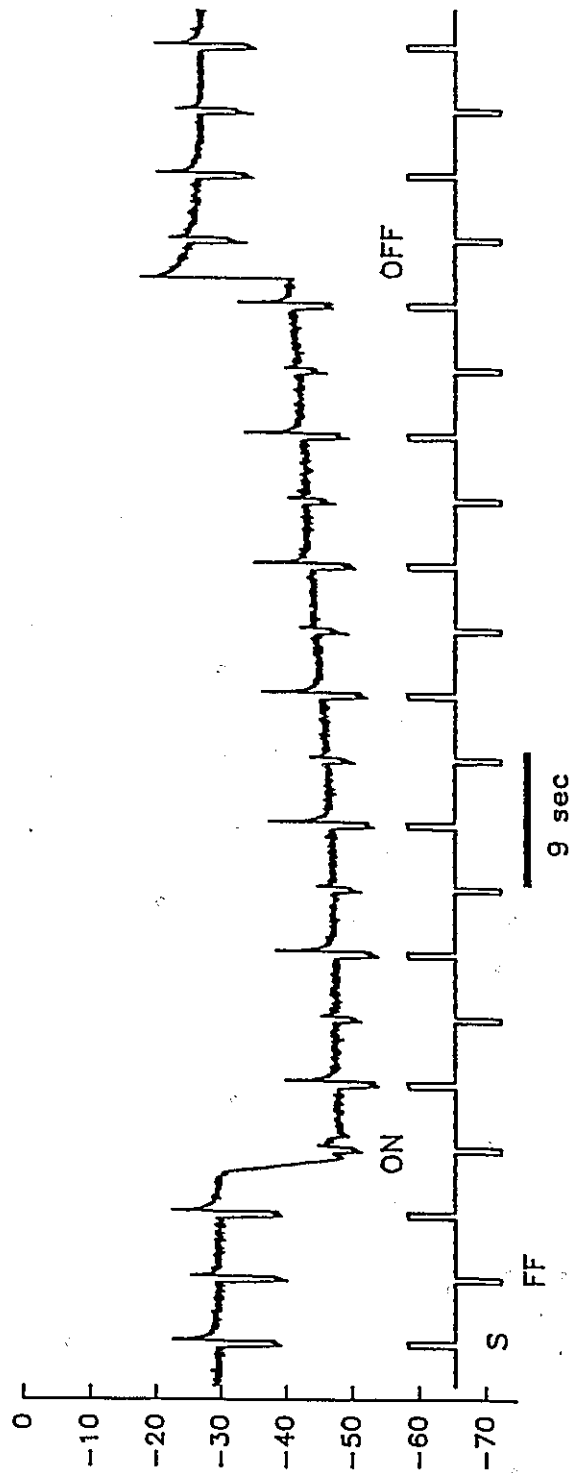


Figure 8 *Second application of background illumination to a light-sensitized retina.* Intracellular recordings from a horizontal cell in the same retina, but later, from which the cell in Fig. 6 was recorded. During background illumination (ON) the horizontal cell hyperpolarized but subsequently depolarized during the period of background illumination. During background illumination the response to slit stimuli (S) was much greater than the response to full-field (FF) stimuli. After background illumination (OFF) the cell depolarized beyond the dark resting membrane potential prior to background illumination but then hyperpolarized to a steady level somewhat (< 5 mV) depolarized compared to the dark resting membrane potential prior to background illumination. After background illumination the response to both slit and full-field stimuli were somewhat reduced but the response to slit stimulus was greater than the response to full-field stimulus, an effect that became more apparent 15-30 sec. after termination of background illumination (see Fig. 9D). Slit intensity $-2.2 \log I_0$, full-field intensity $-3.7 \log I_0$, background intensity $-2.5 \log I_0$. Stimulus time length was 350 msec and ordinate indicates membrane potential in mV.



also occurred in light-sensitized retinas. An increase in the light onset hyperpolarizing transient was observed during and after application of background illumination (Fig. 7B, 7C and 9B, 9C, solid arrows). This change in waveform also persisted after exposure to background illumination (Fig. 9D, solid arrow). The depolarizing transient at light offset (open arrows) decreased during and after background illumination (Fig. 7B, 9B, 9C) and, in particular, in the responses to slit stimuli.

5.4 Responses to annular stimuli

In addition to comparisons of horizontal cell responses to slit and full-field stimuli in light-sensitized retinas, responses to annuli stimuli were also measured. A decrease in the response to annulus stimuli was noted when light-sensitized retinas were first exposed to background illumination (Fig. 10) before a persistent change in the response to full-field compared to slit stimuli was observed (Fig. 4). Even though the amplitude of the response to slit and full-field stimuli remained unchanged after the termination of background light (Fig. 4) there was a reduction in the response to annulus stimuli in the same cell (Fig. 10). After background illumination the dark resting membrane potential was somewhat (< 5 mV) depolarized. The waveform of the responses to annulus stimuli showed an increase of a light onset hyperpolarizing transient after background illumination (Fig. 10, solid arrows).

Figure 9 Changes in horizontal cell waveform during a second application of background illumination to a light-sensitized retina. Details of the response waveform of the horizontal cell depicted in Fig. 8. (A) Prior to background illumination the response to full-field stimuli (FF) was somewhat greater than the response to the slit (S). (B) During background illumination the response to slit stimuli was greater than the response to full-field stimuli. The hyperpolarizing transient at light onset was increased in the response to full-field stimulus (filled arrow). The depolarizing transient at light off was increased in the response to slit stimuli (large open arrow) but decreased in the response to full-field stimuli (small open arrow). (C) After background illumination the response to slit stimuli increased relative to full-field stimuli. The increase in the transient depolarization at light off in response to the slit (large open arrow), seen during background (B), did not persist but the decrease in this transient in response to full-field stimulus did (small open arrows). The hyperpolarizing transient at light onset was increased in response to both stimuli, but especially in response to the latter (filled arrow). (D) 30 sec after the record shown in C demonstrates that the persistent increase in the response to slit stimuli, relative to full-field stimuli, and shows an increase in the hyperpolarizing transient at light onset in response to both slit and full-field stimuli (filled arrow).

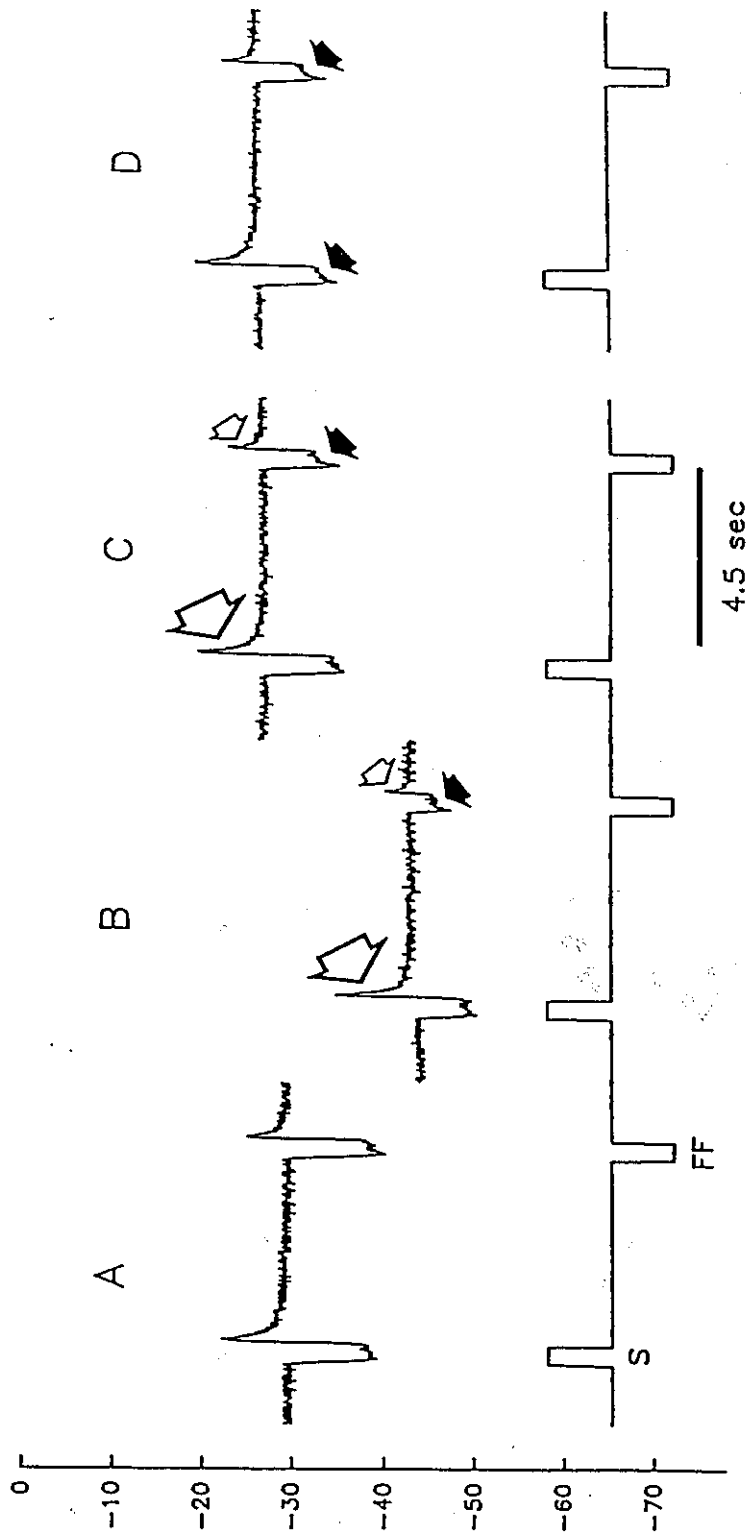
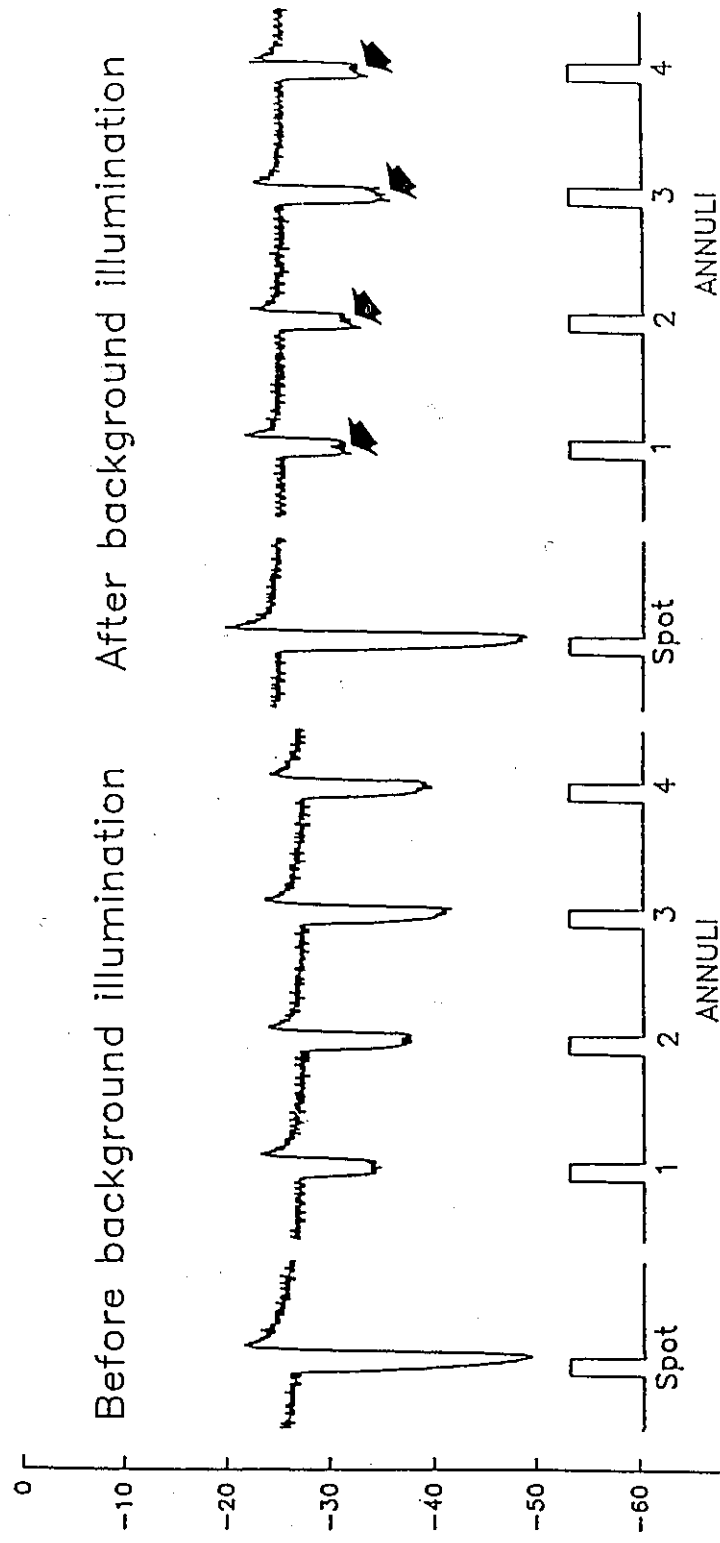


Figure 10 Horizontal cell responses to annuli stimuli in a light-sensitized retina with modest exposure to background illumination. Recordings from the same horizontal cell as shown in Fig. 4 showing the response to a large spot and annulus stimuli before and after application of background illumination. After background illumination the response to annuli stimuli was decreased but the response to the large spot was not. After background illumination the dark resting membrane potential was somewhat (< 5 mV) depolarized. The waveform of the responses to annuli stimuli showed the presence of a hyperpolarizing transient at light onset after background illumination (arrows). Spot size was 7.5 mm diameter. Annuli were (inner diameter/outer diameter) 0.75 mm/1.5 mm (Annulus 1), 2.5 mm/3.75 mm (Annulus 2), 2.5 mm/5.25 mm (Annulus 3) and 3.0 mm/8.0 mm (Annulus 4). The intensity of both spot and annulus stimuli was $-2.4 \log I_0$. Background illumination was $-2.5 \log I_0$. Spot and annulus stimuli time length were 350 msec and the ordinate indicates the potential of the cell in mV.



5.5 Statistics

In the 15 horizontal cells from dark-adapted retinas studied, 12 showed reduced responses to full-field stimuli relative to slit stimuli during the first application of background illumination (eg. Fig. 4). Each of the 3 that did not show a change in response to slit and full-field stimuli did show evidence of light-sensitization. In each of the retinas from which these three cells came, but during recordings from different horizontal cells, application of additional background illumination ultimately decreased the response to full-field stimuli relative to slit stimuli. No horizontal cell showed a persistent change in the response to full-field stimuli relative to slit stimuli after only a single 1 min exposure to background illumination. Of the 12 cells that showed a decrease in the response to full-field stimuli relative to slit stimuli during background illumination, 7 ultimately showed a persistent decrease after the background illumination was turned off (eg. Figs. 6, 8). In each of the 5 cells in which a persistent change of full-field and slit response after background illumination could not be demonstrated prior to losing the cell, subsequent recordings from the same retina, but in different horizontal cells, ultimately showed a persistent decrease in the response to full-field stimuli relative to slit stimuli after background illumination.

The effect of background illumination on the ratio of slit/full-field response of horizontal cells is summarized in Fig. 11. In dark-adapted retinas background illumination decreased horizontal cell slit/full-field response ratio (Fig. 11A). In light-sensitized retinas background illumination increased the horizontal cell slit/full-field response ratio during background illumination (Fig. 11B). In horizontal cells with a significant light history this increase persisted after the background illumination was turned off indicating that the retina was light-adapted (Fig. 11B, squares). In some horizontal cells the increase observed during background illumination did not persist indicating that the retina was still in the light-sensitized state (Fig. 11B, circles). In these retinas further application of background illumination resulted in horizontal cell responses with a persistent increase in slit/full-field response ratio.

5.6 Changes of horizontal cell waveform

Analysis of the hyperpolarizing and depolarizing transients of responses to slit and full-field stimuli revealed a pattern of change of these responses during and after background illumination. An illustration of the parameters measured in this analysis is shown in Fig. 12.

The amplitude of the hyperpolarizing transients as a proportion of total horizontal cell response are given in Table 1. Prior to background illumination, the proportion of the maximum response contributed by the hyperpolarizing

Figure 11 Summary of changes in slit response/full-field response ratio before, during and after application of background illumination. (A) Application of background illumination to a dark-adapted retina, as illustrated in Fig. 1, decreased ($p=0.012$) the slit/full-field response ratio after background illumination. The reduction during background illumination was not statistically significant. (B) Application of background illumination to light-sensitized retinas increased the slit/full-field response ratio only during background illumination (circles, $p<0.001$), as in Fig. 4, or during and after ($p=0.039$) background light (squares), as in Fig. 8. Error bars are standard error of the mean.

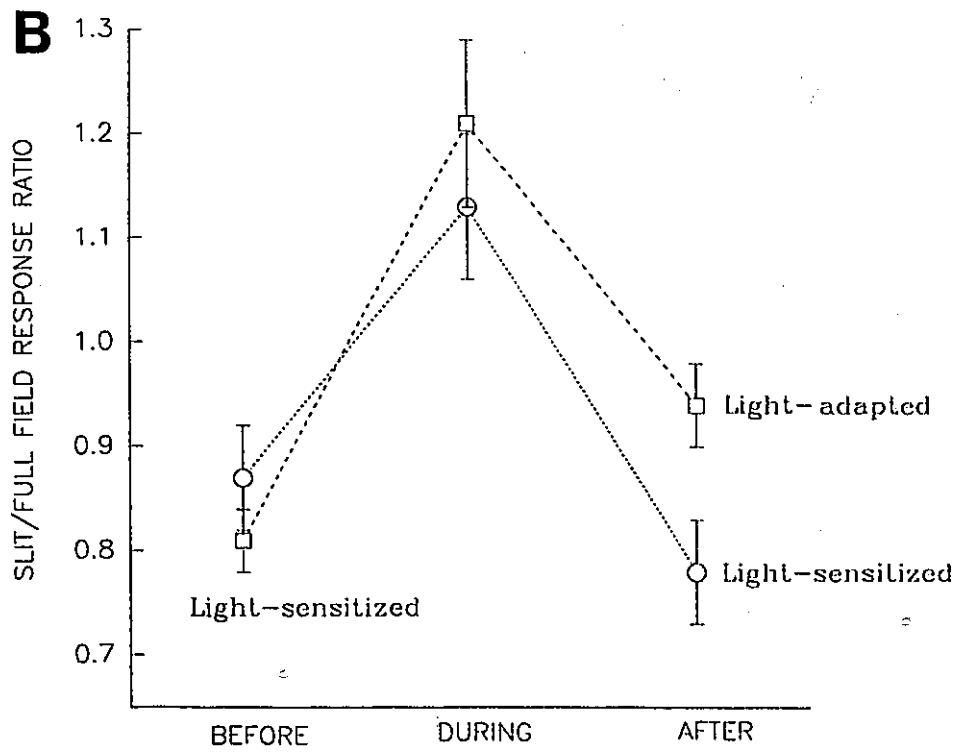
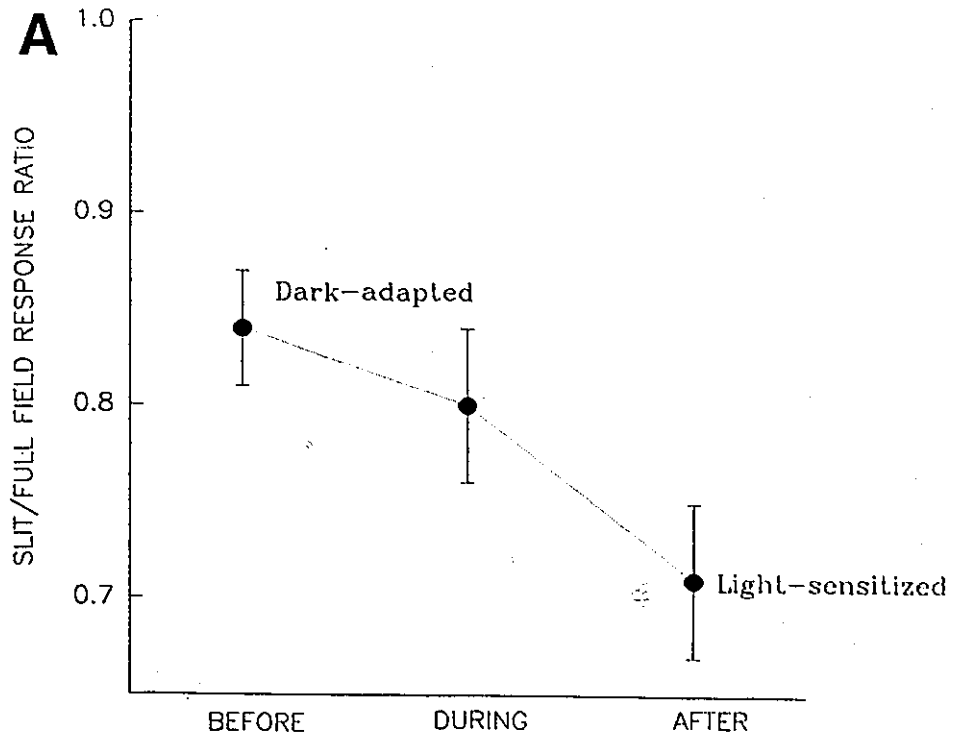
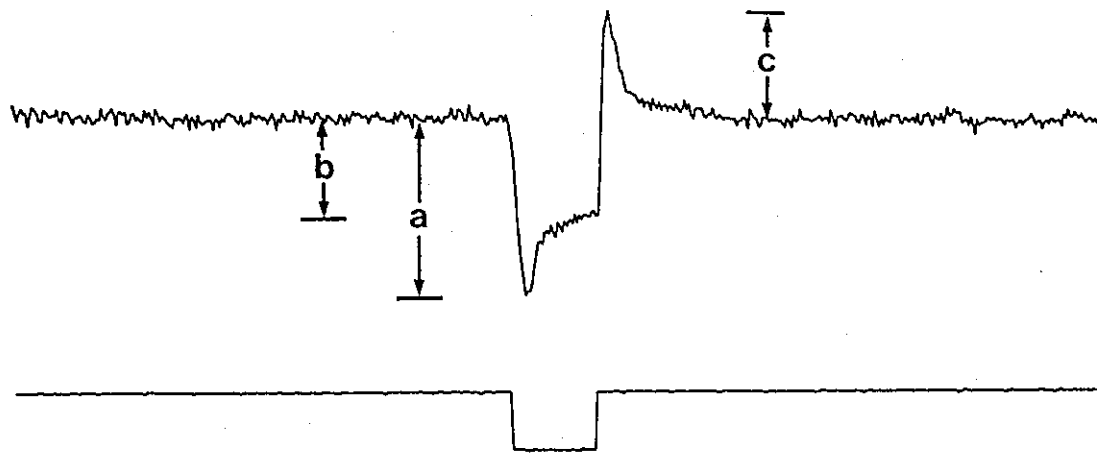


Figure 12 Parameters of horizontal cell waveform analysis as given in Tables 1-3. The size of the hyperpolarizing transient as a proportion of total horizontal cell response (Table 1) was calculated from $(a-b)/a$ for full-field and slit stimuli, before, during and after application of background illumination. The change of depolarizing transient (Table 2) was calculated by $c_{\text{during}}/c_{\text{before}}$ and $c_{\text{after}}/c_{\text{before}}$ for both full-field and slit stimuli responses. The amplitude of depolarizing transients relative to maximum horizontal cell response amplitude (Table 3) was calculated from c/a for full-field and slit stimuli responses, before, during and after application of background illumination. Tables 1-3 report means \pm standard errors from the results of several cells. Statistical analysis was performed using oneway analysis of variance.



12

transient was 8% of the total response to full-field stimuli and 7% of the response to slit stimuli. During background illumination these proportions rose to 41%, in the case of responses to full-field stimuli, and 14% in the responses to slit stimuli. After the background illumination was turned off, the proportions were 19% for full-field stimuli and 10% for slit stimuli. This analysis shows that the hyperpolarizing transient at light onset increased during and after background illumination and, in particular, in the responses to full-field stimuli. The change in this transient was not necessarily correlated with changes in the response to slit and full-field stimuli as it was observed both during light-sensitization (Fig. 3), during background illumination, and after light-adaptation (Figs. 5, 7, 9).

The change of the amplitude of the depolarizing transient during and after background illumination is shown in Table 2. The depolarizing transient responses at light off in responses to full-field stimuli decreased on average by 66% during background illumination. However, this change was found to be proportional to the change of the response amplitude of the horizontal cell (Table 3). The increase of the ratio of the transient response/maximum horizontal cell response, from 0.34 to 0.47, was not significant ($p=0.077$). After background illumination, no consistent change in the depolarizing transient at light off in the response to full-field stimuli

Table 1 -- Amplitude of hyperpolarizing transient as a proportion of total horizontal cell response (see Fig. 12).

	before background illumination	during background illumination	after background illumination
full-field stimulus	0.08 ± 0.02 n=14	0.41 ± 0.03 n=14 (p<0.001)	0.19 ± 0.04 n=12 (p=0.011)
slit stimulus	0.07 ± 0.02 n=14	0.13 ± 0.03 n=14 (p=0.13)	0.10 ± 0.03 n=12 (p=0.42)

Table 2 -- Change of depolarizing transient amplitude (see Fig. 12).

	$C_{\text{during}}/C_{\text{before}}$ background illumination	$C_{\text{after}}/C_{\text{before}}$ background illumination
full-field stimulus	0.66 ± 0.10 n=14	0.99 ± 0.12 n=13
slit stimulus	1.48 ± 0.22 n=15	1.04 ± 0.13 n=13

Table 3 -- Amplitude of depolarizing transients relative to maximum horizontal cell response amplitude (see Fig. 12).

	before background illumination	during background illumination	after background illumination
full-field stimulus	0.34 ± 0.06 n=14	0.47 ± 0.04 n=14 p=0.077	0.33 ± 0.05 n=14 p=0.887
slit stimulus	0.36 ± 0.07 n=13	0.88 ± 0.06 n=13 p<0.001	0.47 ± 0.08 n=13 p=0.276

For each of Table 1-3 the values reported are mean ± standard error. Statistical analysis was performed using oneway analysis of variance.

was noted when compared to such transients before background illumination.

The depolarizing transient in the responses to slit stimuli increased on average by 48% during background illumination (Table 2) and the ratio of depolarizing transient response/slit response amplitude increased greatly, from 0.36 before background illumination to 0.88 during background (Table 3). The increase in the slit response depolarizing transient did not consistently persist after background illumination was extinguished (Tables 2, 3).

6.0 Discussion

In the present study we have evaluated the receptive field properties of horizontal cells before, during and after background illumination. Our findings demonstrate that horizontal cells of dark-adapted fish retina first pass through a state of light-sensitization, where responsiveness and receptive-field size are increased, after exposure to background illumination. Subsequent exposure of such light-sensitized retina to additional background illumination reduces horizontal cell responsiveness and receptive-field size. The latter effect of background illumination has been termed light-adaptation.

Yang, Tornqvist and Dowling (1988a) have demonstrated that after presentation of background illumination to a dark-adapted retina, both responsiveness and receptive-field size increase, resulting in a light-sensitized retina. After it was observed that application of background illumination reduced horizontal cell receptive-field size in retinas presumed to be dark-adapted it was proposed (Baldrige and Ball 1991; Umino, Lee and Dowling 1991) that this reduction was actually observed in light-sensitized retinas rather than dark-adapted retinas and that the background illumination resulted in light-adaptation.

The horizontal cells from dark, light-sensitized or light-adapted retinas have different response and receptive-field size properties (summarized in Table 4).

Table 4 -- Summary of retinal adaptation states and effect on horizontal cell properties

	Receptive-field size	Responsiveness
Dark-adapted	reduced	reduced
Light-sensitized	increased	increased
Light-adapted	reduced	reduced

Our results demonstrate the difference between horizontal cell receptive-field size in light-sensitized and light-adapted retinas. Horizontal cells from dark-adapted retinas had reduced receptive-field size and were light-sensitized by background illumination so that their responsiveness and receptive-field size increased. Additional exposure of these light-sensitized retinas to background illumination reduced horizontal cell receptive-field size. This study showed that in the same retina application of background illumination to dark-adapted retina first increased horizontal cell responsiveness and receptive-field size (light-sensitization) and then, with additional exposure to background illumination, reduced horizontal cell receptive-field size (light-adaptation).

Yang, Tornqvist and Dowling (1988a) have suggested that light-sensitization is due to decreased release of dopamine from interplexiform cells. In the dark-adapted retina, dopamine reduces horizontal cell responsiveness by increasing the sensitivity of a glutamate-gated current (Knapp and Dowling 1987; Liman, Knapp and Dowling 1987) and reduces horizontal cell receptive-field size by a resulting decrease of membrane resistance and because of increased gap junction resistance (Teranishi, Negishi and Kato 1984; Mangel and Dowling 1985; Lasater and Dowling 1985a; 1985b). Therefore, increased responsiveness and receptive-field size in the

light-sensitized retina are consistent with decreased release of dopamine.

Another model has been proposed by Kamermans et al. (1990; Kamermans and Werblin 1992) that suggests that reduced GABAergic autofeedback after light-sensitization increases horizontal cell responsiveness and receptive-field size. In the dark-adapted retina, when GABAergic autofeedback is strong, GABA acts at horizontal cell GABA_A-receptors increasing a Cl⁻-conductance with a reversal potential positive to the dark resting membrane potential. As a result, light-induced changes of glutamate-dependent currents are shunted by the increased Cl⁻-conductance, horizontal cell responsiveness decreased and receptive-field size decreased due to the decreased membrane resistance (R_m).

In our experiments most horizontal cells did not show evidence of light-sensitization after exposure to background illumination. Instead, these cells showed evidence of becoming light-adapted after exposure to background illumination. We suggest that horizontal cells from these retinas were already light-sensitized prior to the first treatment with background illumination as a result of exposure to light during preparation of the retina. Our technique for dark-adapting and preparing the retina under dim red light exposed the retina to minimal light in these procedures. This suggests that the dark-adapted retina can be readily light-sensitized, even by the dim illumination used during retinal

isolation. Another study has also demonstrated that the dark-adapted fish retina is easily light-sensitized (Mangel et al. 1993). The ease with which dark-adapted retinas become light-sensitized suggests that great care must be taken to assure that a retina is in a dark-adapted state. It may be that the only way to be certain that a retina is dark-adapted is to avoid exposure to light completely by dark-adapting the isolated retina, and not just the fish (Mangel et al. 1993).

In our experiments on light-sensitized retinas, exposure to background illumination initially reduced horizontal cell receptive-field size only when background illumination was on and did not persist after background illumination was turned off. Additional background illumination resulted in a reduction of horizontal cell receptive-field size that persisted even after background illumination was turned off. Persistent changes in the receptive-field size of horizontal cells that occur after exposure to background illumination have been reported previously in carp, goldfish (Shigematsu and Yamada 1988; Baldrige and Ball 1991) and mudpuppy (Dong and McReynolds 1991) retinas. Others have reported that background illumination reduces horizontal cell receptive-field size in the presence of background illumination but did not report a persistent reduction after background illumination was turned off (Umino, Lee and Dowling 1991; Weiler and Akopian 1992). However, it is not known if additional exposure to background illumination would have

revealed persistent changes in receptive-field size. Examination of the records from Umino, Lee and Dowling (1991) suggest the development of a persistent change in horizontal cell receptive-field size after the brightest of several exposures to background illumination.

The observed reduction in the receptive-field size of light-sensitized horizontal cells that occurred during background illumination, and later persisted, could be due to either an increase in gap junction resistance (R_i) or a decrease in membrane resistance (R_m) (Lamb 1976). As discussed in the methods, increased R_i can be revealed physiologically by an increase in the response to a small spot or slit stimulus (Piccolino, Neyton and Gerschenfeld 1984; Teranishi, Negishi and Kato 1984) whereas decreased R_m results in a decrease in response to full-field stimuli (Mangel and Dowling 1985; 1987). Changes in the response to annulus stimuli can be due to either or both (Piccolino, Neyton and Gerschenfeld 1984; Dong and McReynolds 1991).

Because the reduction in horizontal cell response amplitude to full-field stimuli was accompanied by a reduction in the response to slit stimulus both during and after exposure to steady background illumination, the observed reduction in horizontal cell receptive-field size is consistent with a reduction in R_m . Although the responses to slit stimuli increased relative to the responses to full-field stimuli, the absolute responses to slit stimuli did not

increase. Shigematsu and Yamada (1988) also found that background illumination increased the response to a slit relative only relative to the response to a slit displaced from the centre of the horizontal cell receptive field. A relative increase in slit response, compared to full-field response, may be due only to a change in R_m in combination with decreased horizontal cell responsiveness and may not involve any increase of R_i .

We have previously suggested (Baldrige and Ball 1991) that the effect of background illumination on horizontal cell receptive-field size was due, at least in part, to an increase in R_i because Lucifer yellow dye coupling was reduced by background illumination. Furthermore, a change in R_i during and after background illumination cannot be ruled out on the basis of electrophysiological data because the desensitization of horizontal cell responsiveness during and after background illumination does not permit a comparison of the response to a slit before background illumination. In the isolated retina, cone photoreceptors are bleached by bright light resulting in a desensitization of horizontal cells (Norman and Perlman 1990). This may explain why, in the present study, a decreased horizontal cell response to full-field stimuli was accompanied by a decreased response to slit stimuli even though the relative decrease to full-field stimuli was greater. It is therefore not possible to determine if a light-induced increase in gap junction resistance (R_i), which

would result in an increase of the response to slit stimuli, was masked by the bleaching of the cones that desensitizes horizontal cell responsiveness.

An additional finding of the present work is the observation of a light-dependent persistent reduction of horizontal cell responses to annuli stimuli that precedes a persistent decrease in the response to full-field stimuli. The annuli stimuli used may detect changes in receptive-field size before slit or full-field stimuli because annuli stimuli is sensitive to changes in both R_1 and R_2 . Therefore, small changes in either R_1 or R_2 may not be detected by comparing horizontal cell responses to slit or full-field stimuli alone. Because annuli stimuli did detect a change in receptive-field size before slit and full-field stimuli, it is likely that the changes detected by annuli during and after presentation of background illumination were due to small rather than large changes of R_1 or R_2 , or both.

It is not possible to determine if the changes in horizontal cell receptive-field size observed during and after background illumination are due to the same mechanism. The persistent change in horizontal cell receptive field-size following background illumination was always accompanied by a reduction of horizontal cell receptive-field size while the background illumination was on. This may indicate that the decrease of horizontal cell receptive-field by background illumination proceeds by a common mechanism that first

decreases receptive-field size during background illumination and later even when the background illumination is turned off.

The mechanism underlying the reduction in horizontal cell receptive-field size during and after background illumination is unknown. It is possibly mediated by the release of a neurotransmitter or neuromodulator that remains to be identified. Alternatively, the change in horizontal cell receptive-field size during background illumination may be a reflection of decreased non-synaptic R_m during hyperpolarization (Trifonov, Chailahian and Byzov 1971; Trifonov, Byzov and Chailahian 1974). This is opposite to the change in synaptic R_m that increases with light as cone transmitter release is reduced (Byzov and Trifonov 1968; Trifonov 1968). However, in the present study the membrane potential of horizontal cells returned to pre-background illumination levels or was only slightly depolarized after background illumination. Therefore, a change in voltage-dependent non-synaptic R_m is unlikely to be the mechanism of the persistent change in horizontal cell receptive-field size. This may indicate that the change in horizontal cell receptive-field size during and after background illumination are not, as suggested above, mediated by a common mechanism.

The persistent reduction of horizontal cell receptive-field size caused by background illumination in turtle and mudpuppy retina is thought to be due to an increase in R_i mediated by dopamine release (Dong and McReynolds 1991; Weiler

and Akopian 1992). In the fish retina, however, dopamine release is thought to reduce horizontal cell receptive-field size in dark-adapted horizontal cells, also believed to be due to an increase in R_1 (Mangel and Dowling 1985; Yang, Tornqvist and Dowling 1988b; Tornqvist, Yang and Dowling 1988). Two studies of fish retina have demonstrated that dopamine is not involved in the mechanism that reduces horizontal cell receptive-field size during and after exposure of light-sensitized retinas to background illumination (Baldrige and Ball 1991; Umino, Lee and Dowling 1991).

The reason for the apparent difference between the action of dopamine in different vertebrate retinas is unknown but may be related to the different types of neurons containing dopamine in these different vertebrate retinas. In fish, dopamine is contained within interplexiform cells (Ehinger, Falck and Laties 1969; Sarthy and Lam 1979; Marc 1982; Yazulla and Zucker 1988) that make specific contacts onto horizontal cells. In turtle and mudpuppy retina, however, dopamine is contained within amacrine cells that do not make specific contacts with horizontal cells (Adolph, Dowling and Ehinger 1980; Nguyen-Legros et al. 1985b). In the latter case it has been suggested that dopamine affects horizontal cells by diffusing from the inner retina to the outer retina (Piccolino, Neyton and Gerschenfeld 1984; Witkovsky, Stone and Besharse 1988). The relationship between these morphological

differences and apparent functional differences remains to be determined.

The waveform of the horizontal cell response to full-field and annulus stimuli changed during and after exposure to background illumination. Prior to background illumination the waveform of dark-adapted horizontal cells was slow and the cell gradually hyperpolarized while the test stimulus was on. Cells from light-sensitized retinas responded to the same stimuli with a faster hyperpolarization followed by a gradual depolarization while the stimulus was on. This response pattern gave the horizontal cell the appearance of having a transient hyperpolarizing response at light onset, particularly in response to full-field stimuli. Increases in the hyperpolarizing transient persisted in the responses to full-field stimuli after background illumination was turned off. These changes in waveform were noted in both light-sensitized and light-adapted retinas.

A transient hyperpolarization at stimulus onset has also been identified in waveform of H2 horizontal cells by Weiler and Wagner (1984) in carp retina exposed to background illumination. They argued that the observed waveform changes reflected increased feedback to green cones by H1 horizontal cells. The feedback from H1 horizontal cells would depolarize the green cone, increase transmitter release and depolarize H2 horizontal cells. The transient response at stimulus onset would be due to the synaptic delay between the

hyperpolarization from red cones and the depolarizing feedback from H1 horizontal cells.

It has been suggested that horizontal cell membrane hyperpolarization, as would occur in the presence of background illumination, could lead to increased horizontal cell to cone feedback (Kaneko and Tachibana 1986; Umino, Watanabe and Hashimoto 1989). It is possible, therefore, that the reduction of horizontal cell responses to full-field and annuli stimuli during background illumination is associated with increased horizontal cell to cone feedback. However, such membrane potential-dependent changes should not persist after background illumination is turned off and the horizontal cell membrane depolarizes. Furthermore, the appearance of the hyperpolarizing transient at light onset was noticeable after light-sensitization, when horizontal cell receptive-field size was increased. Therefore, either the observed hyperpolarizing transients do not represent feedback or increased feedback is not responsible for the reduction of horizontal cell responses to full-field stimuli during and after background illumination.

Depolarizing transients at light offset were observed in responses to both slit and full-field stimuli. These transients, in the responses to full-field stimuli, were found to be proportional to the response amplitude. In responses to slit stimuli the depolarizing transients increased during background illumination but not after. Off-overshoot

responses have also been observed in the horizontal cells of the tiger salamander retina and were shown to originate in a subpopulation of rods that provide input to horizontal cells in these retinas (Wu 1988). The origin and significance of the depolarizing transients at light offset in fish cone horizontal cells is not known.

In summary, the results of this study demonstrate that background illumination increases the responsiveness and receptive-field size of dark-adapted fish horizontal cells. After application of background illumination to a dark-adapted retina, the retina is light-sensitized. The effect of background illumination may increase horizontal cell R_h and decrease horizontal cell R_l , possibly due to decreased release of dopamine in the retina. Alternatively, reduced GABAergic autofeedback in light-sensitized retina could explain both increased responsiveness and receptive-field size due to changes in R_h alone. This study further demonstrated that exposure of light-sensitized retinas to additional background illumination reduced horizontal cell responsiveness and receptive-field size. Changes in horizontal cell receptive-field size during background illumination might be due to a mechanism sensitive to the hyperpolarization of the horizontal cell during light exposure. However, the mechanism that results in a persistent change in horizontal cell receptive-field size after background illumination is unlikely to depend on such voltage-dependent mechanisms. Therefore, it may be

there exists a light-dependent mechanism, perhaps a messenger, that produces the persistent change in horizontal cell receptive-field size.

IV. Effect of norepinephrine on [³H]-dopamine release
and horizontal cell receptive-field size
in the goldfish retina

1.0 Preface and significance to thesis

This chapter (IV) and the next (V) contain the findings of two studies investigating the possibility that horizontal cell receptive-field size is regulated by norepinephrine or epinephrine. The first study, on the effects of norepinephrine, was presented at the 1991 Association for Research in Vision and Ophthalmology meeting (Baldrige et al. 1991) and has been published by Brain Research (Baldrige, Tomasic and Ball 1993). The second study was presented at the 1992 Association for Research in Vision and Ophthalmology meeting (Ball and Baldrige 1992) and has been published in NeuroReport (Baldrige and Ball 1993).

Both of these studies were prompted by the identification of putative adrenergic neurons in the goldfish retina (Ball and Baldrige 1991, 1992). In particular, a new type of interplexiform cell immunoreactive for phenylethanolamine-N-methyltransferase (PNMT), the synthesizing enzyme for epinephrine, was identified. Confocal microscopy demonstrated that the processes of the PNMT-immunoreactive interplexiform cell ramified in the outer plexiform layer, between the somata of horizontal cells and

the photoreceptor synaptic terminals. Therefore, this cell could provide an adrenergic input to horizontal cells. The possibility of an adrenergic innervation of horizontal cells raised the possibility that an adrenergic transmitter might be involved in the light-dependent reduction of horizontal cell receptive-field size described in Chapters II and III.

The results of the two studies reported here (Chapter IV and V) do not support the hypothesis that horizontal cell receptive-field size is regulated by an adrenergic system. The anatomical findings leave open the possibility that some other aspect of horizontal cell function, or photoreceptors, bipolar cells or pigment epithelium, might be mediated by an adrenergic system. The effect of exogenous norepinephrine and epinephrine on horizontal cells are likely due to the action of these compounds as agonists of dopamine receptors. Furthermore, pharmacological experiments did not suggest the presence of adrenoreceptors on horizontal cells. Because the effects of norepinephrine and epinephrine on horizontal cells were blocked by dopamine receptor antagonists, a treatment that does not block the effect of background illumination, it is not possible to conclude that an adrenergic neurotransmitter system is involved in the effects of background illumination on horizontal cells.

2.0 Summary

Norepinephrine increased the release of pre-loaded [³H]-dopamine from goldfish retinas. Pharmacological studies suggested that the norepinephrine-induced [³H]-dopamine release was due to an exchange mechanism between norepinephrine and pre-loaded [³H]-dopamine. Norepinephrine also depolarized and reduced the receptive-field size of horizontal cells in goldfish retinas. The action of norepinephrine on horizontal cells was probably not due to the release of endogenous dopamine because the effect of norepinephrine was not abolished in retinas in which all dopaminergic neurons had been destroyed by prior treatment with 6-hydroxydopamine. The pharmacology of the effect of norepinephrine on horizontal cells suggested that it was due to an agonist action of norepinephrine acting at horizontal cell dopamine receptors. It is still unclear whether endogenous norepinephrine is a regulator of dopamine release in the fish retina. Consequently, the function of the putative norepinephrine-containing amacrine cells of the fish retina remains to be elucidated.

3.0 Introduction

Dopamine, the predominant catecholamine neurotransmitter in the teleost retina (DaPrada 1977; Weiler, Kolbinger and Kohler 1989), has been localized to 'I1' interplexiform cells (Ehinger, Falck and Laties 1969; Yazulla and Zucker 1988). Several studies have identified different mechanisms that regulate dopamine (DA) release (Kato et al. 1982; O'Connor, Zucker and Dowling 1987; Harsanyi and Mangel 1992; Rashid, Baldrige and Ball 1993) and the effects of DA on several retinal cell types, including horizontal cells, photoreceptors and pigment epithelium, have been investigated (Teranishi, Negishi and Kato 1984; Dearry and Burnside 1986a; Mangel and Dowling 1987; Weiler et al. 1988a).

The role of another catecholamine, norepinephrine (NE), in the teleost retina has been subject to disagreement. Some studies have reported little (DaPrada 1977) or no (Osborne and Ghazi 1988) NE in teleost retina, while other studies using high pressure liquid chromatography (HPLC) have identified significant amounts of NE (Jaffe et al. 1987; Dearry and Burnside 1989). The synthesizing enzyme for NE, dopamine- β -hydroxylase (DBH), has been identified in fish retina biochemically (Jaffe, Urbina and Drujan 1991) and a subset of amacrine cells has been found to be DBH-immunoreactive (DBH-IR) (Ball and Baldrige 1991). An example of a DBH-IR amacrine cell in the goldfish retina is shown in Fig. 1. The reported presence of α_2 - and β -adrenoreceptors in teleost

retina (Dearry and Burnside 1988; O'Connor, Kropf and Dowling 1989) also suggests a possible role for an adrenergic neurotransmitter system in these retinas.

Because the neurites of DBH-IR amacrine cells (Fig. 1a) overlap with those of dopaminergic 'I1' interplexiform cells (Fig. 1b), we tested the hypothesis that NE might regulate DA release in the retina by studying the effect of NE on the release of preloaded [³H]-DA from isolated superfused goldfish retinas. To determine if NE might affect horizontal cell receptive-field size, possibly by regulating endogenous DA release, we also investigated the action of NE on horizontal cell receptive-field size in the isolated, superfused goldfish retina.

4.0 Materials and Methods

4.1 Animals

Adult goldfish (*Carassius auratus*) (Tropical Fish Supply, Fort Erie, ON), 15-20 cm in length, were maintained on a 12 hr light/12 hr dark lighting cycle. Experiments were carried out near the middle of the light phase of the cycle.

4.2 Tissue preparation for [³H]-dopamine uptake and release

Fish that were first dark-adapted for 1 hr were decapitated in dim light, both eyes removed, hemisected and the retinas isolated in Ringer solution. The Ringer solution consisted of 80 mM NaCl, 3.5 mM KCl, 22.7 mM NaHCO₃, 2.4 mM MgSO₄, 2.3 mM CaCl₂, 10 mM D-glucose, 10 mM HEPES bubbled continuously with 95% O₂, 5% CO₂, maintained at 20°C, and at pH

Figure 1 Overlays of confocal micrographs showing DBH- (A) and TH-IR (B) somata (arrows) in the inner nuclear layer (INL) of the goldfish retina. Neurites from both cells are located in S1 and S5 of the inner plexiform layer (IPL) (open arrows).

DBH

TH

1A

1B

-25µA

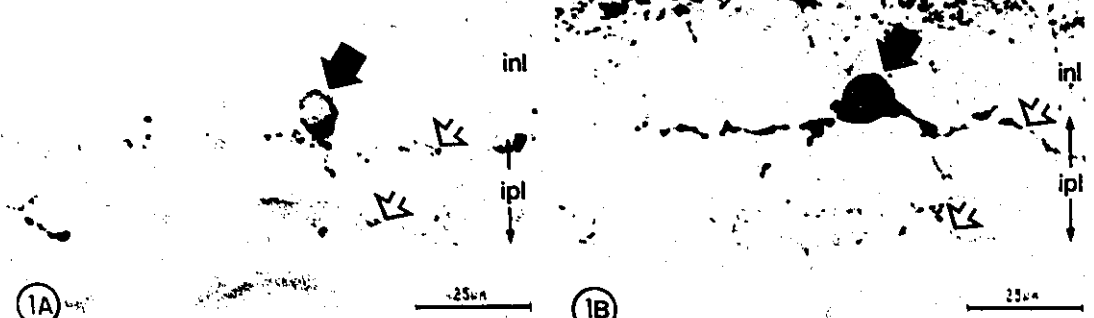
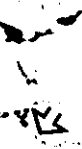
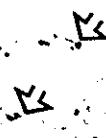
25µA

inl

ipl

inl

ipl



7.2. 0.1 mM ascorbic acid, an anti-oxidant, and 15 μ M pargyline (Sigma), a monoamine oxidase inhibitor, were added to the Ringer to prevent degradation of [3 H]-DA during incubation and superfusion. This Ringer was used in order to be consistent with previous studies of [3 H]-DA release in the fish retina (Kato et al. 1982; O'Connor et al. 1986; O'Connor, Zucker and Dowling 1987; Rashid, Baldrige and Ball 1993). Both retinas were then incubated in the dark in Ringer containing 0.5 μ M [3 H]-dopamine (#NET-094, NEN Dupont, Markham, ON) for 15 min, washed several times in Ringer, incubated in Ringer for an additional 15 min and then placed in a filter holder (Nucleopore 13 mm, Pleasanton, CA) between nylon mesh. The retinas were superfused continuously with Ringer (2.5 - 3.5 ml/min) for at least 20 min in the dark prior to beginning each treatment.

4.3 Treatments affecting [3 H]-dopamine release

Test drugs dissolved in Ringer were delivered to the superfusion chamber by a computer-controlled multiport valve (Rheodyne Inc., Cotati, CA). The effect of the following drugs on [3 H]-dopamine release were tested: NE (Arterenol, Sigma), phentolamine, propranolol (α - and β -adrenoreceptor antagonists), clonidine, isoproterenol (α - and β -adrenoreceptor agonists), nomifensine (DA uptake inhibitor) (Research Biochemicals Inc. (RBI), Natick, MA). Antagonists were used at concentrations that exceeded reported rat brain IC_{50} values for the receptor to which they were directed

(Bylund and Snyder 1975; Seeman 1981; Tsukamoto et al. 1984). The effect of NE was also studied in calcium-free Ringer by replacing CaCl_2 with 2.3 mM MgCl_2 and adding 0.5 mM EGTA. In most experiments there was at least one treatment with Ringer containing elevated potassium ($[\text{K}^+]_o = 40$ mM, balanced by a corresponding reduction of $[\text{Na}^+]_o$) to evaluate the robustness of $[\text{}^3\text{H}]\text{-DA}$ release in each retina studied.

4.4 Measurement of $[\text{}^3\text{H}]\text{-dopamine}$ release

The superfusate was collected using a fraction collector every 1 min, dissolved in Scintiverse (Fisher Scientific, Don Mills, ON), and radioactivity measured by scintillation counting. Relative changes of $[\text{}^3\text{H}]\text{-DA}$ release were determined by taking the difference between the peak release and the baseline.

4.5 Tissue preparation for intracellular electrophysiology

A detailed description of the isolated superfused goldfish retina preparation is described in detail elsewhere (Baldrige and Ball 1991). Briefly, goldfish were dark adapted for 1 hr, and then killed by decapitation followed by pithing. Retinas were isolated and superfused with Ringer consisting of 110 mM NaCl , 2.5 mM KCl , 20 mM NaHCO_3 , 0.1 mM MgSO_4 , 0.7 mM CaCl_2 , 20 mM D-glucose, bubbled continuously with 95% O_2 , 5% CO_2 , maintained at 20°C and pH 7.2. This Ringer was used to be consistent with Ringer used in other electrophysiological studies of teleost horizontal cells.

(Mangel and Dowling 1987; Baldrige and Ball 1991; Harsanyi and Mangel 1992).

4.6 Light stimuli

Stimuli were presented to the vitreal side of the retina using a dual-beam optical bench under computer control. Illumination was provided by a 250-W tungsten-halogen bulb (Rotron, Woodstock, NY). The electrode was aligned to the optical axis of the stimuli using an infrared stimulus and an infrared imaging camera (Electrophysics, Nutley, NJ). The unattenuated intensity of white light stimuli was 3×10^{-10} W/ μm^2 . Intensities used in these experiments are reported as \log_{10} differences of the unattenuated intensity.

4.7 Intracellular recordings

Glass microelectrodes were pulled on a Flaming/Brown puller (Sutter Instruments, Novato, CA), filled with 2.5M KCl, and had resistances of about 60 M Ω . The microelectrodes were advanced into the retina using a piezoelectric microdrive (Burleigh Inchworm, Burleigh Instruments, Fishers, NY) under computer control. Horizontal cell responses were identified and categorized according to their characteristic waveform, large receptive-field size, pattern of responses to chromatic stimuli and depth of the penetrating microelectrode. All recordings reported here were from H2 horizontal cells. Horizontal cell responses were amplified, viewed on an oscilloscope, and recorded on magnetic tape for later playback or plotting.

4.8 Measurement of horizontal cell receptive-field size

The receptive-field size of horizontal cells was measured by comparing the amplitude of horizontal cell responses to alternate slit and full-field stimuli. This comparison was used to differentiate between possible changes in gap junction resistance and membrane resistance during the experiments. When horizontal cell gap junction resistance increases and the input resistance of the horizontal cell network increases, larger responses to slit stimuli are recorded (Piccolino, Neyton and Gerschenfeld 1984). Horizontal cell responses to full-field stimuli are unaffected by changes in gap junction resistance because all the cells in the syncytium are equipotential under full-field illumination (Mangel and Dowling 1987). However, if responses to both slit and full-field stimuli decrease, this indicates a change in membrane resistance rather than gap junction resistance.

To assess horizontal cell receptive-field size changes, responses to a centred $250 \mu\text{m} \times 7000 \mu\text{m}$ slit were compared to full-field illumination. The intensity of both stimuli was adjusted to produce responses of equal amplitude (approximately 10 mV) to white-light stimuli.

In some experiments horizontal cell receptive-field size was measured by translating the slit away from the receptive-field centre and the response amplitude measured in $250 \mu\text{m}$ increments. The use of a long, narrow slit permitted the determination of a one-dimensional length constant (λ)

because the current spreads only orthogonal to the slit and not parallel to its long axis under which horizontal cells are equipotential. Estimates of λ were made by finding the best fit for the exponential function $e^{-r/\lambda}$ to the data by adjusting the value of λ (where r is the slit translation distance from the position of maximum response amplitude).

4.9 Treatments affecting horizontal cell receptive-field size

Drugs, and their delivery, were the same as described for [³H]-DA release experiments. Additional drugs used were the α_2 -adrenoreceptor antagonist yohimbine (RBI) and the D2>D1 DA receptor antagonist haloperidol (McNeil Laboratories, Stouffville, ON). As in the release experiments, antagonists were applied at concentrations that exceeded reported IC₅₀ values in brain (Bylund and Snyder 1975; Seeman 1981; Tsukamoto et al. 1984).

The effect of NE on horizontal cell receptive-field size was also studied in retinas in which the endogenous source of DA had been destroyed by giving two 50 μ g intraocular injections of 6-hydroxydopamine (6-OHDA, Sigma) on two subsequent days, 8 to 14 days prior to the experiments. The selectivity and effectiveness of this lesioning protocol has been previously demonstrated (Baldrige and Ball 1991).

5.0 Results

5.1 Effects of NE on [³H]-dopamine release

Although the basal release of [³H]-DA differed from experiment to experiment, the plots of [³H]-DA release

presented in the figures are representative examples of typical experiments. Each treatment was replicated 2-3 times and each showed similar relative changes in the release pattern.

Both 3 μM and 30 μM NE increased the release of [^3H]-DA in the presence or absence of calcium (Fig. 2). Treatment with 3 μM NE increased [^3H]-DA release by about 1.6X and 30 μM NE increased release by about 2.5X in Ringer containing 2.3 mM calcium (Fig. 2, filled circles) or in calcium-free Ringer containing 0.5 mM EGTA (Fig. 2, open circles). In contrast, raising $[\text{K}^+]$ from 3.5 mM to 40 mM increased the release of [^3H]-DA only when calcium was present in the Ringer (Fig. 2).

The observed increase in [^3H]-DA release induced by 30 μM NE was not blocked by either the α -adrenergic antagonist phentolamine (100 μM) (Fig. 3) or the β -adrenergic antagonist propranolol (100 μM) (Fig. 4). Furthermore, the release of [^3H]-DA was not increased by 100 μM clonidine, an α -adrenoreceptor agonist (Fig. 5). However, there was a slight increase (about 1.4X) in the release of [^3H]-DA by 100 μM isoproterenol, a β -adrenoreceptor agonist (Fig. 5).

The release of [^3H]-DA induced by 30 μM NE was reduced by 50% in the presence of 100 μM nomifensine, a blocker of the DA uptake system (Fig. 6).

These experiments demonstrate that NE induces the release of pre-loaded [^3H]-DA from interplexiform cells. This release was calcium independent and not mediated by an

Figure 2. Release of pre-loaded [^3H]-DA from superfused goldfish retinas in normal (closed circles) and calcium-free (open circles) Ringer. A 2 min pulse of 40 mM [K^+], increased the release of [^3H]-DA in Ringer containing 2.3 mM calcium but had no effect in calcium-free Ringer. 2 min pulses of either 3 μM or 30 μM NE, however, increased the release of [^3H]-DA either in the presence or absence of calcium. Hatched lines below abscissa indicate when the Ringer contained raised [K^+], or NE, as indicated in the graph.

Figure 3. Stimulation of [^3H]-DA release after a 2 min pulse of 30 μM NE was not blocked by prior treatment with 100 μM phentolamine (PHENT), an α -adrenoreceptor antagonist.

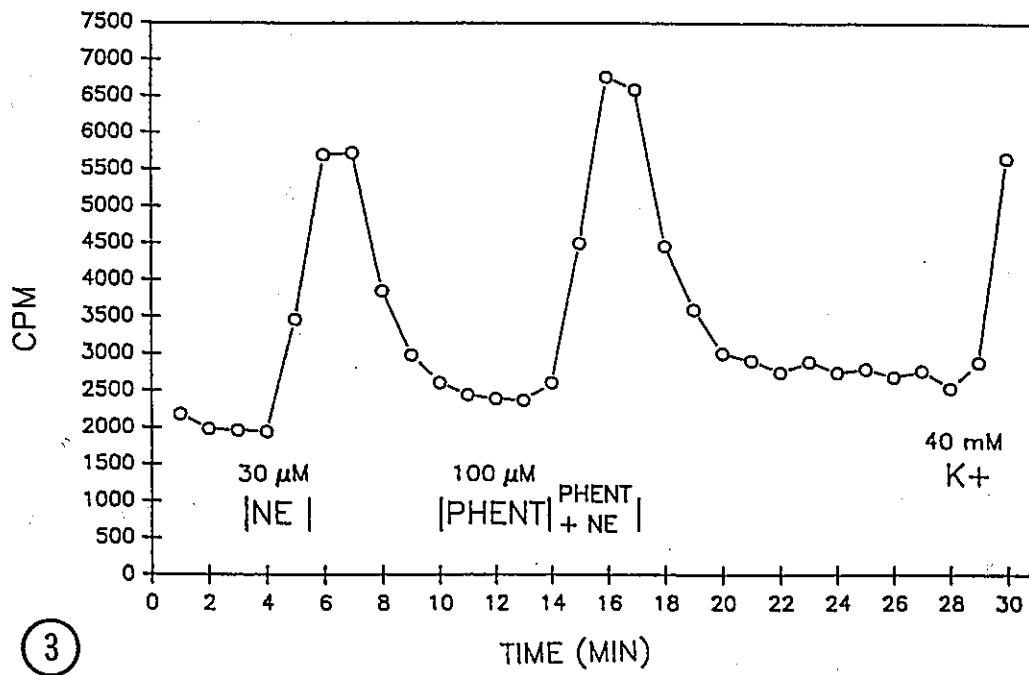
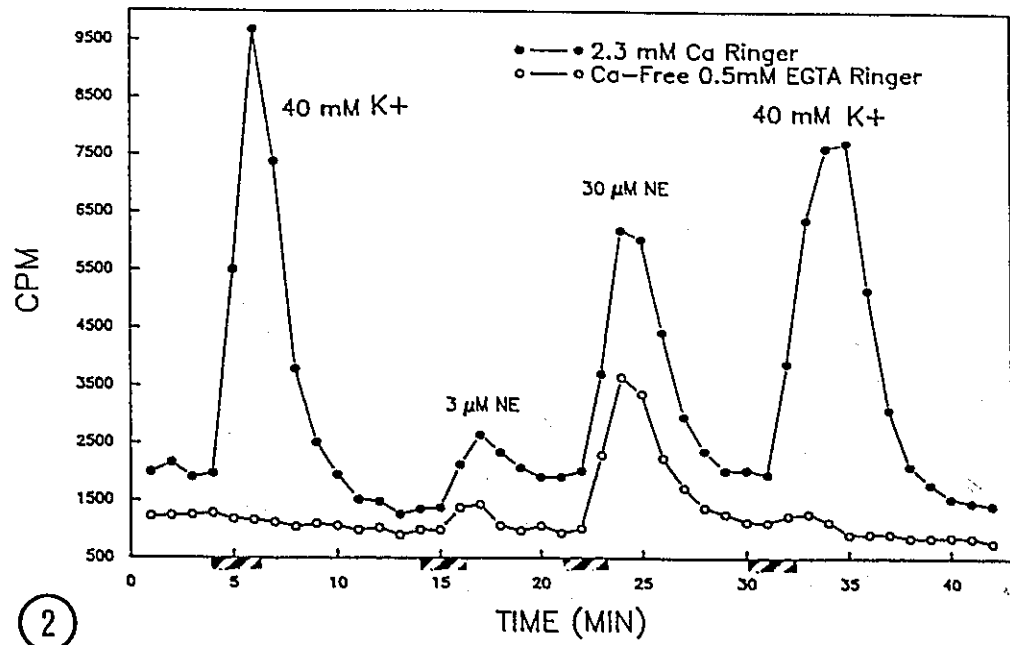
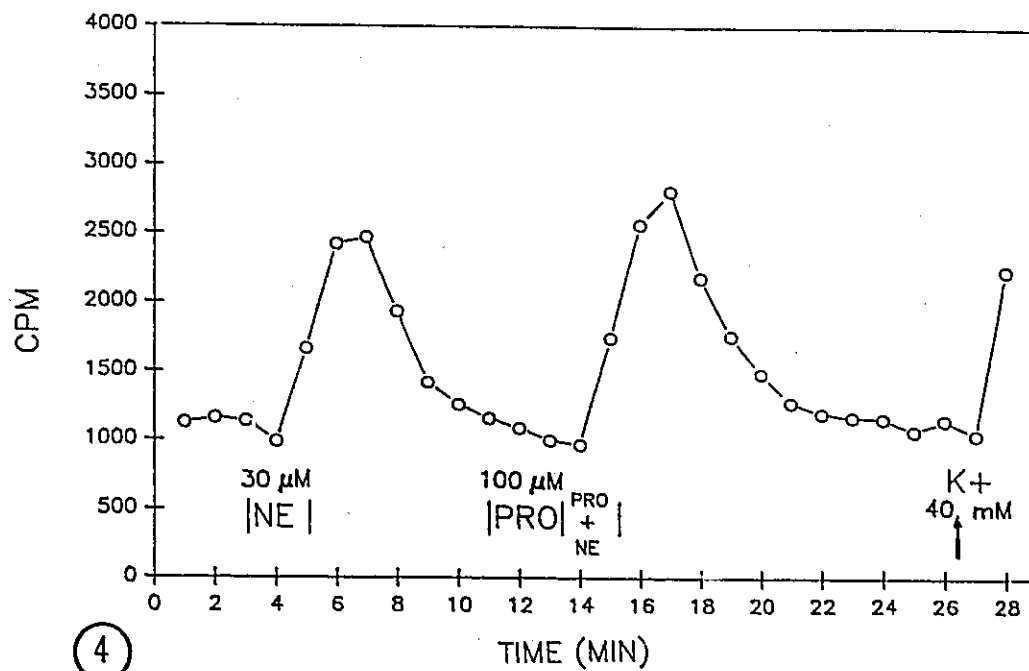
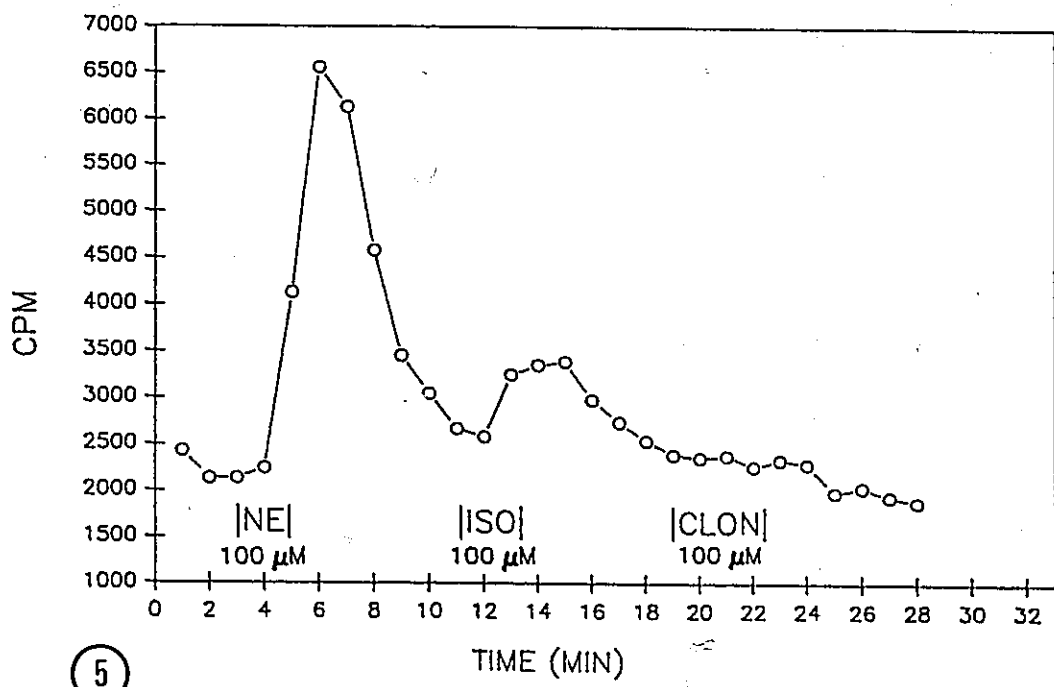


Figure 4. Stimulation of [³H]-DA release after a 2 min pulse of 30 μM NE was not blocked by prior treatment with 100 μM propranolol (PROP), a β-adrenoreceptor antagonist.

Figure 5. Pulses of 100 μM α- and β-adrenoreceptor agonists, clonidine (CLON) and isoproterenol (ISO), respectively, did not mimic the effect of 100 μM NE on [³H]-DA release. Although clonidine had no effect, a slight increase in [³H]-DA release following treatment with isoproterenol was observed.

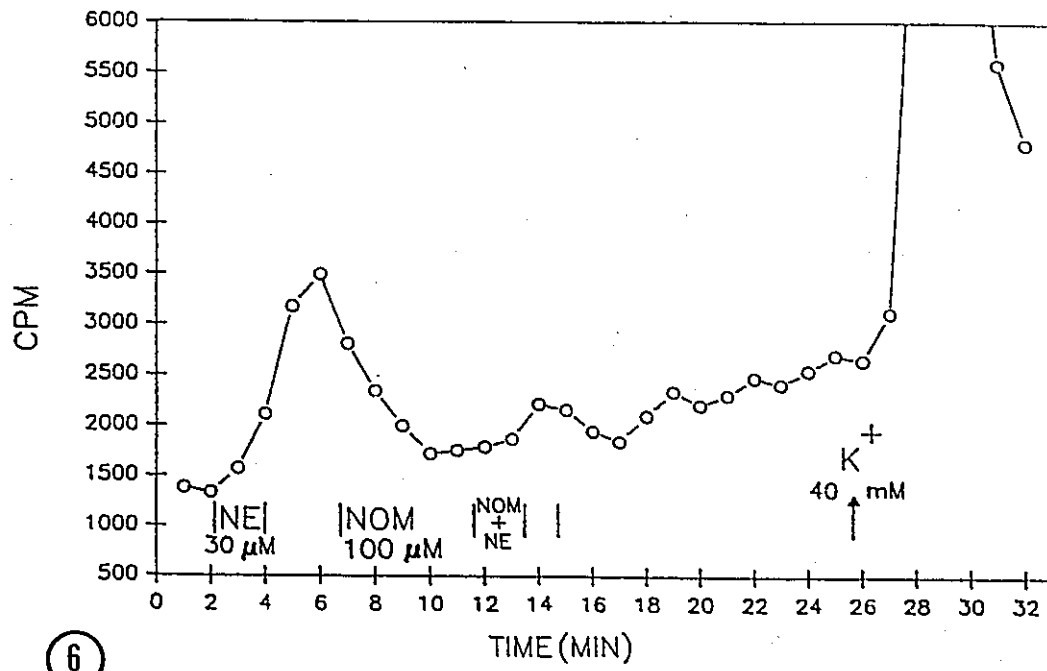


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Figure 6. Treatment with 100 μ M nomifensine (NOM), a DA-uptake blocker, reduced [3 H]-DA release expected after a 2 min pulse of 30 μ M NE. After treatment with nomifensine there was a gradual increase in [3 H]-DA release prior to treatment with 40 mM [K^+].



6

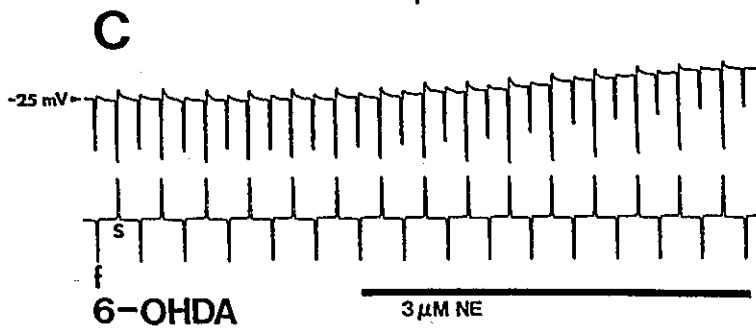
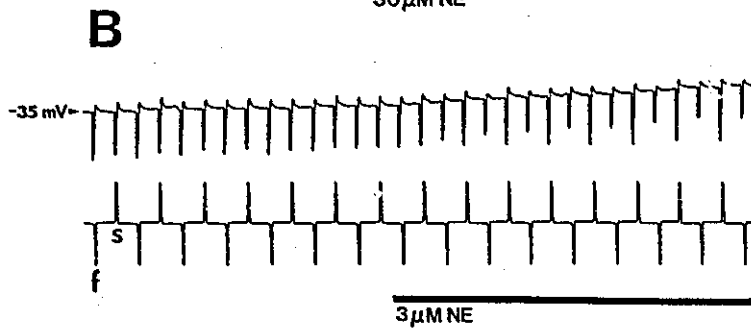
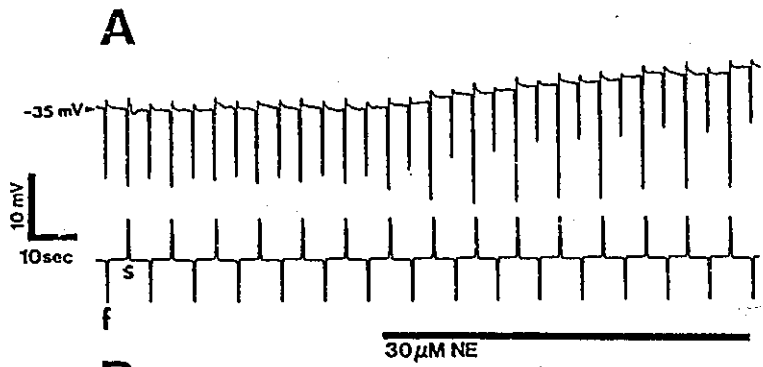
adrenergic receptor, implicating an exchange mechanism between NE and pre-loaded [³H]-dopamine. To determine if NE could release DA from cells that were not pre-loaded, the effect of NE on a known target of dopaminergic interplexiform cell innervation, horizontal cells (Ehinger, Falck and Laties 1969; Piccolino, Neyton and Gerschenfeld 1984; Teranishi, Negishi and Kato 1984; Mangel and Dowling 1987; Yazulla and Zucker 1988) was studied.

5.2 Effects of NE on receptive-field size of horizontal cells

Both 3 μ M and 30 μ M NE depolarized the dark resting membrane potential and reduced the receptive-field size of horizontal cells (Fig. 7). The intensities of centred slit and full-field stimuli were first adjusted to produce responses of approximately equal amplitude. A change in horizontal cell receptive-field size was revealed when there was an increase in the response to the slit (Fig. 7, s) and a decrease in the response to the full-field stimulus (Fig. 7, f) (Piccolino, Neyton and Gerschenfeld 1984; Mangel and Dowling 1987). After 1 min of either 3 μ M (Fig. 7A) or 30 μ M (Fig. 7B) NE, the amplitude of the response to the slit was increased and the response to the full-field stimulus was decreased. This effect was observed in 17 of 18 H2 horizontal cells we recorded.

Recovery from the effects of NE was not observed during the typical length of time (5 - 10 min) that intracellular recordings were made from the same horizontal cell. However,

Figure 7. Both (A) 3 μM and (B) 30 μM NE reduced the receptive-field size of horizontal cells in isolated, superfused goldfish retina. NE depolarized horizontal cell membrane potential by 5-10 mV, decreased the response to full-field stimulus (f) (intensity -5.0 log in (A), -3.7 log in (B)) and increased the response to a 250 μm x 7000 μm slit stimulus (s) (intensity -3.0 log). This change in response properties indicates a reduction in horizontal cell receptive-field size (Mangel and Dowling 1987; Piccolino, Neyton and Gerschenfeld 1984). (C) 3 μM NE had the same effect on horizontal cell receptive-field size in retinas where dopaminergic interplexiform cells were lesioned by 6-OHDA (slit intensity -3.0 log, full-field intensity -5.0 log).



7

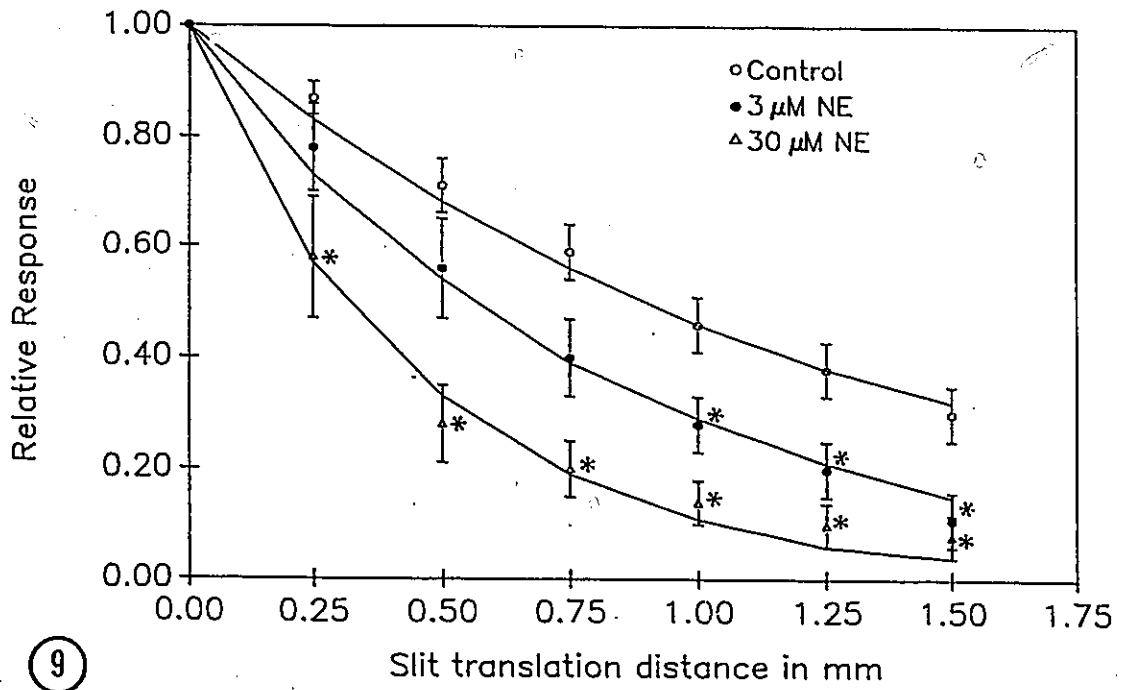
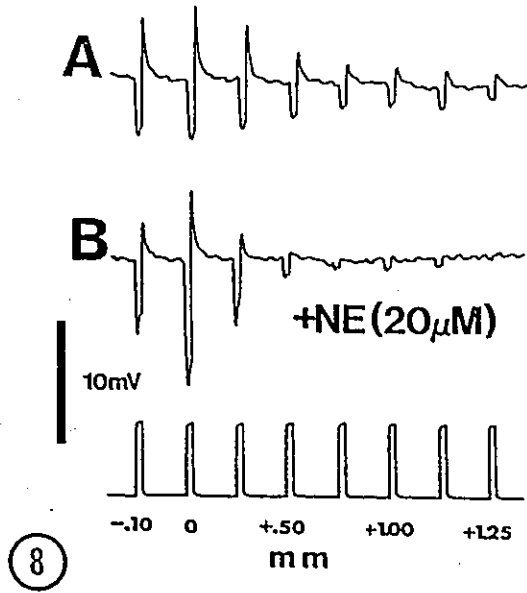
30 - 40 min of Ringer wash did appear to reverse the effects of NE since newly impaled horizontal cells had similar response amplitudes to the slit and full-field stimuli after the wash.

Changes in the receptive-field size of horizontal cells caused by NE treatment were also observed in experiments where a slit of light was translated across the retina (Fig. 8). The decrease in the response to the slit with increasing distance from the receptive-field centre was greater after a 4 min treatment with 20 μM NE than before NE treatment. The change in horizontal cell receptive-field size was quantified (Fig. 9) by determining the decay in response with increasing translation distance for several cells before (n=14) (Fig. 9, open circles) and after (n=6) a 2 min treatment with 3 μM (Fig. 9, filled circles) or 30 μM NE (Fig. 9, triangles). The calculated values of λ were $\lambda=1.30$ mm in control retinas, $\lambda=0.80$ mm after 3 μM NE, and $\lambda=0.45$ mm after 30 μM NE.

The effect of NE on horizontal cells was similar to the previously reported effects of dopamine on horizontal cells (Piccolino, Neyton and Gerschenfeld 1984; Mangel and Dowling 1987). Because NE was shown to stimulate [^3H]-DA release it is possible that the effects of NE on horizontal cells might be via an increase in endogenous DA release from dopaminergic interplexiform cells. Such an indirect effect of NE on horizontal cells is also suggested by the finding that DBH-IR amacrine cells do not contact horizontal cells directly, but

Figure 8. 20 μM NE reduces horizontal cell receptive-field size measured by translating a 250 μm x 7000 μm slit (intensity -3.4 log). (A) Responses of a horizontal cell to a slit stimulus as it was translated away from the centre of the receptive-field in 0.25 mm steps. Responses were still observed after the slit was translated 1.25 mm. (B) Responses of the same horizontal cell to a translated slit after a 4 min pulse of 20 μM NE. A response is no longer apparent at 1.25 mm and the amplitude of the response at the centre of the receptive field (0 mm) is increased after NE treatment.

Figure 9. Mean relative response to slit stimuli in several horizontal cells from control (open circle) (n=14), 3 μM NE treated (closed circles) (n=6), and 30 μM NE treated (open triangles) (n=4) retinas. Relative response is the response at each point the slit was translated divided by the response at the centre of the receptive field. Curves of the function $V_{(x)}=V_{(0)}e^{-x/\lambda}$ were fitted to the data by determining suitable values of λ . $\lambda_{\text{control}}=1.30$ mm, $\lambda_{3\mu\text{M NE}}=0.80$ mm, $\lambda_{30\mu\text{M NE}}=0.45$ mm. Error bars= S.E.M. *= significantly different values from control retina at $\alpha=0.05$ as determined by t-test.



are in a position to contact dopaminergic interplexiform cells (Fig. 1) (Ball and Baldrige 1991). To test whether the effect of NE on horizontal cell receptive-field size was due to release of DA, receptive-field sizes of control and NE-treated horizontal cells were measured in retinas in which dopaminergic neurons had been destroyed by prior treatment with 6-OHDA (Fig. 7c).

In each of 3 cells studied, the effect of NE on horizontal cells in 6-OHDA-lesioned retinas was indistinguishable from the effect of NE on horizontal cells in unlesioned retinas. This result suggests that the effect of NE on horizontal cell receptive-field size may not be mediated by release of endogenous DA. Therefore, the possibility that NE might have a direct effect on horizontal cells was investigated.

The increased spot and decreased full-field responses produced by 3 μM NE were not blocked by the β -adrenergic antagonist propranolol (50 μM) (Fig. 10A) in each of 4 cells studied. Although the increased spot response produced by 3 μM NE was blocked by the α -adrenergic antagonist phentolamine (50 μM) (Fig. 10B) in all 3 cells tested, the decrease in the response to full-field stimuli and the observed membrane depolarization was not blocked by phentolamine (50 μM). Clonidine (30 μM), an α -adrenergic receptor agonist, had no effect on horizontal cell receptive-field size or responsiveness (Fig. 11B) in either of 2 cells studied. While

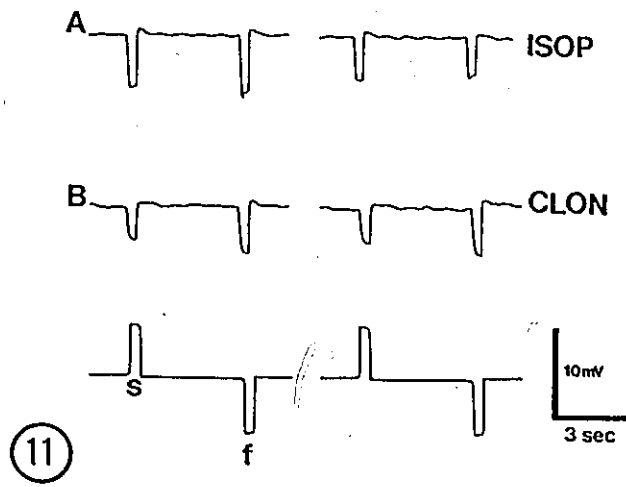
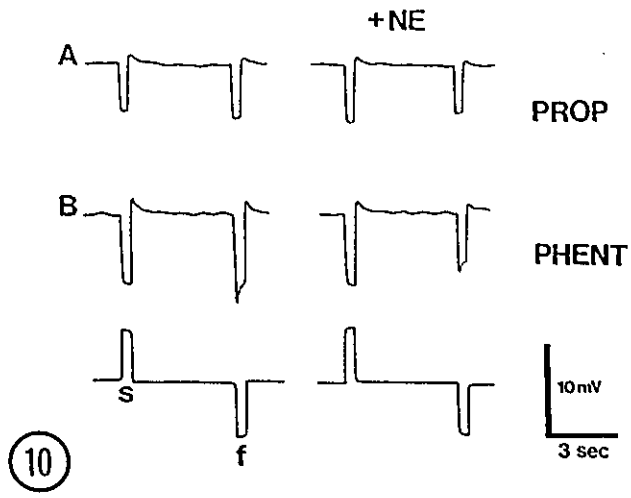
30 μM isoproterenol, a β -adrenergic receptor agonist, reduced horizontal cell responses to a full-field stimulus, it also reduced the response to a slit stimulus in 3 cells studied (Fig. 11A) suggesting that isoproterenol decreased horizontal cell responsiveness but not receptive-field size.

The specific α_2 -adrenoreceptor antagonist yohimbine (50 μM), blocked the effect 3 μM NE on horizontal cell receptive-field size (Fig. 13A) in each of 4 cells studied, but it was also found that 50 μM yohimbine also blocked the effect of 6 μM DA (Fig. 13B) in each of 3 cells studied. These results suggest that the antagonism of NE by yohimbine was due to its action on DA receptors. Although yohimbine is reported to be a selective α_2 -adrenoreceptor antagonist (Goldberg and Robertson 1983) there are also some indications that yohimbine may also act as a DA receptor antagonist (Scatton, Zivkovic and Dedek 1980; Dearry and Burnside 1988).

Haloperidol (50 μM), a DA receptor antagonist, blocked the effect of 3 μM NE on horizontal cells (Fig. 12) in each of three cells studied. No change in the horizontal cell response to either slit or full-field stimuli was noted after treatment with 3 μM NE when treatment was preceded by a 2 min pulse of haloperidol. The blocking of the effect of NE on horizontal cells by haloperidol suggests that NE may have been acting at DA receptors.

Figure 10. (A) The reduction of horizontal cell receptive-field size by 3 μM NE was not blocked by the β -adrenoreceptor antagonist propranolol (PROP) but (B) was affected by the α -adrenoreceptor antagonist phentolamine (PHENT). The left panel of each illustrates the response to slit (s) and full-field (f) stimuli after a 2 min treatment with 50 μM antagonist, the right panel shows the responses to the same stimuli after a 2 min pulse of 3 μM NE in the presence of the antagonist. Prior to application of NE the response to full-field stimuli was greater than the response to the slit. After treatment with NE the response to the slit increased in the presence of propranolol but not in the presence of phentolamine. The response to full-field stimuli decreased in both cases. Intensity of slit: -3.5 log; of full-field: -5.0 log. Membrane potential prior to NE was -35 mV, after NE -30 mV.

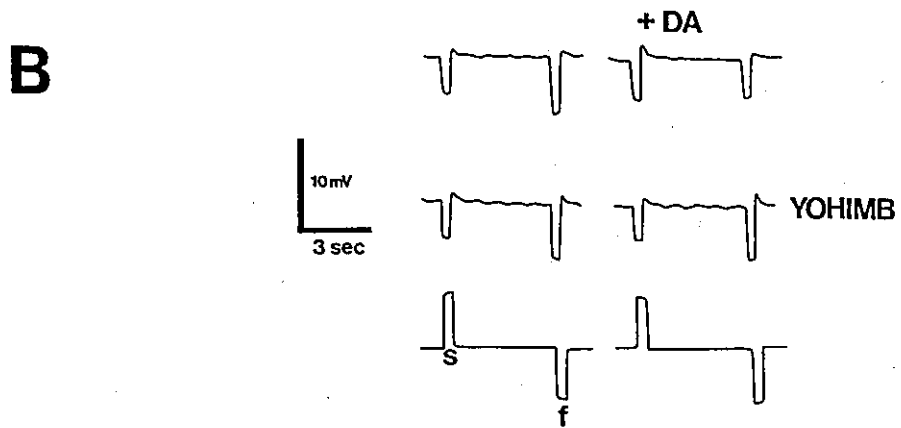
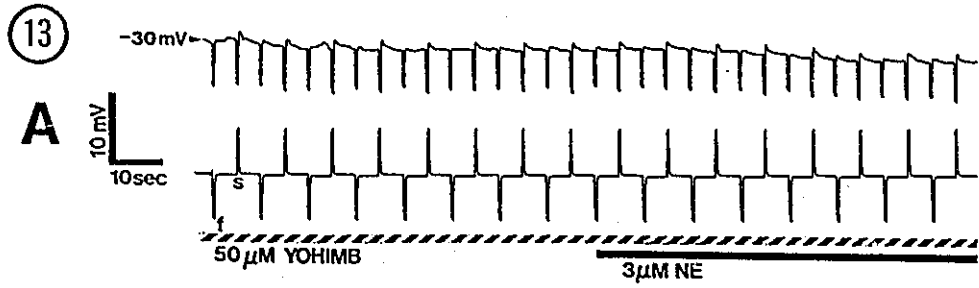
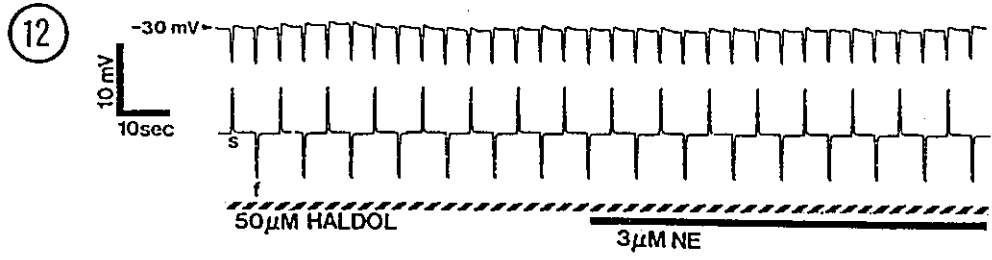
Figure 11. The β - and α -adrenoreceptor agonists (A) isoproterenol (ISO) and (B) clonidine (CLON), respectively, did not mimic the effect of NE on horizontal cell receptive-field size. A 2 min pulse of 30 μM isoproterenol reduced the response to both slit and full-field stimuli and did not markedly increase the response to slit stimuli relative to response to full-field stimuli. Isoproterenol depolarized the dark resting membrane potential from -35 mV to -30 mV. A 2 min pulse of 30 μM clonidine did not affect responsiveness to either slit or full-field stimuli and did not alter the



membrane potential (-40 mV). In both (A) and (B) the left panel illustrates responses prior to agonist treatment, the right panel illustrates the responses after agonist treatment. Intensity of slit: -3.0 log; of full-field -5.0 log.

Figure 12. The dopamine receptor antagonist haloperidol (HALDOL) blocked the effect of 3 μ M NE on horizontal cell receptive-field size. Treatment with NE was preceded by a 2 min pulse of 50 μ M HALDOL. The responses to slit stimuli (-3.0 log) or full-field stimuli (-5.0 log) were not affected by treatment with 3 μ M NE nor was the membrane depolarized.

Figure 13 (A) The α_2 -adrenoreceptor antagonist yohimbine (YOHIMB) blocked the effect of 3 μ M NE on horizontal cell receptive-field size. The responses to slit stimuli (-3.0 log) or full-field stimuli (-5.0 log) were not affected by treatment with 3 μ M NE nor was the membrane potential depolarized when preceded by a 2 min pulse of 50 μ M yohimbine. (B) Yohimbine (YOHIMB) also blocked the effect of 6 μ M dopamine (DA) on horizontal cell receptive-field size. In the absence of yohimbine (UPPER PANEL) a 2 min pulse of DA depolarized the horizontal cell membrane potential from -35 mV to -30 mV, increased the response to slit (s) stimuli (-3.3 log) and decreased the response to full-field (f) stimuli (-5.0 log). In the presence of yohimbine (LOWER PANEL) no change in the membrane potential or the response to either slit (-3.3 log) or full-field (-5.0 log) stimuli were noted.



6.0 Discussion

These studies demonstrate that NE can cause the release of [³H]-dopamine in the goldfish retina. Because this action of NE was not blocked by either α - or β -adrenergic antagonists but was blocked by a dopamine uptake inhibitor, it is likely that the observed release was due to displacement of intracellular dopamine after uptake of NE by the dopamine uptake system, rather than a synaptic action mediated by adrenergic receptors. The findings that the NE-induced release of dopamine was Ca⁺⁺-independent, and that unlabelled dopamine can also induce the release of pre-loaded [³H]-dopamine, are consistent with this hypothesis. Evidence from brain slice and synaptosomal preparations suggests that the dopamine uptake system has an affinity for NE as well as other phenylethylamines (Holz and Coyle 1974; Horn 1976; Rateri et al. 1977; Rateri et al. 1978; Ross, 1976), and that such uptake can induce the release of pre-loaded [³H]-dopamine or other phenylethylamines (Heikkila, Orlansky and Cohen 1975; Rateri et al. 1977; Rateri and Levy 1978) via an exchange mechanism. The affinity of the dopamine uptake system for phenylethylamine derivatives may also explain why isoproterenol, a phenylethylamine-like adrenergic agonist, induced some release of [³H]-dopamine while clonidine, an imidazole, did not. Other studies of the effects of NE on dopamine release have yielded conflicting results. In fish retina Kato et al. (1982) also reported that NE caused a

calcium-independent release of [³H]-dopamine, while O'Connor et al. (1986) reported no effect of NE on [³H]-dopamine release. Our results support the findings of Kato et al. (1982), that NE stimulates release of [³H]-dopamine, and in addition suggests that this release is due to an exchange mechanism rather than receptor-mediated.

It remains to be determined if NE can stimulate release of endogenous dopamine via such a mechanism. Our attempts to observe the effect of NE on endogenous dopamine release using HPLC were not successful because the dopamine peak was obscured by the large peak of exogenously applied NE, which is near the same location on HPLC chromatograms. Therefore, changes in horizontal cell receptive-field size, known to be affected by dopamine (Baldrige and Ball 1991; Harsanyi and Mangel 1992; Mangel and Dowling 1987; Negishi and Drujan 1979; Piccolino, Neyton and Gerschenfeld 1984; Weiler et al. 1988a), were used as a measure of endogenous dopamine release.

Although NE mimicked the effects of dopamine on HC receptive-field size, this does not appear to be due to stimulation of dopamine release, since the effect was equally strong in retinas in which all dopaminergic neurons had been destroyed by pre-treatment with 6-hydroxydopamine. The 6-hydroxydopamine lesioning protocol used in the present study has been shown to result in a 99.9% reduction of retinal dopamine (Baldrige and Ball 1991), and it is unlikely that the persistence of the NE effect in 6-hydroxydopamine lesioned

retinas is due to a compensatory up-regulation of dopamine receptors since dopamine-induced cAMP production is not increased in fish retinas at comparable times after lesioning with 6-hydroxydopamine (Watling, Parkinson and Dowling 1982).

The finding that NE mimics the effect of dopamine on horizontal cells but does not appear to stimulate dopamine release suggests that NE may act directly on horizontal cell dopamine receptors, or at adrenergic receptors which have a similar effect. An action at dopamine receptors seems more likely, since the effect of NE was blocked by haloperidol and by the specific D₁ dopamine receptor antagonist SCH23390, but was not significantly reduced by either α - or β -adrenergic antagonists. Haloperidol is 10 - 100 times more effective at dopamine receptors than at either type of adrenergic receptor (Bylund and Snyder 1975; Seeman 1981; Tsukamoto et al. 1984) and NE can bind to D₁ dopamine receptors with an affinity about one tenth as great as dopamine (Seeman 1981). Although the effect of NE on horizontal cells was slightly reduced by the α -adrenergic antagonist phentolamine, this does not necessarily indicate an action at adrenergic receptors since phentolamine has also been reported to have some effect on dopamine receptors (Seeman 1981). The effect of NE on horizontal cells was also blocked by the α -adrenergic antagonist yohimbine, but this effect could also be attributed to an action at dopamine receptors because yohimbine also blocked the effect of exogenous dopamine on horizontal cells.

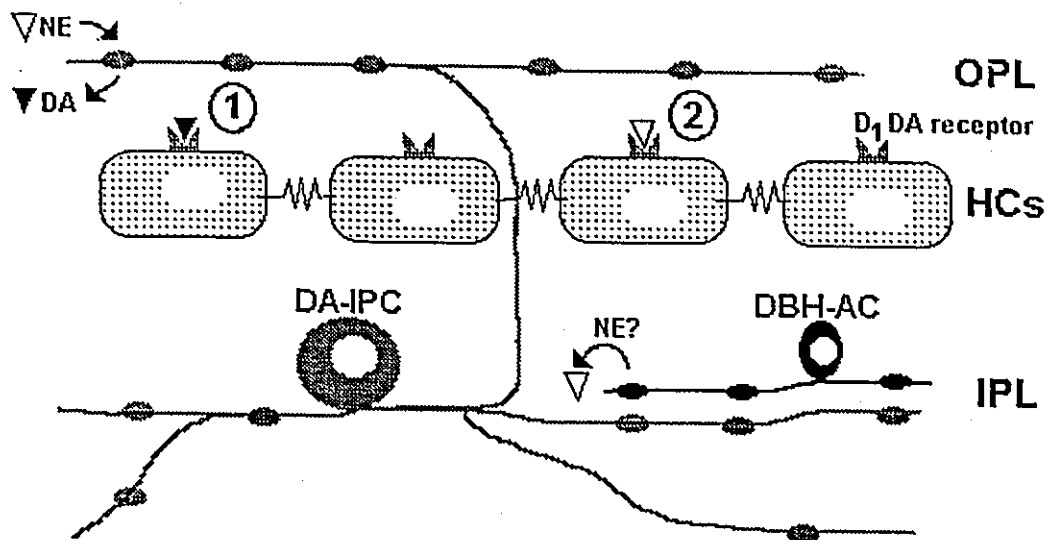
An action of NE on adrenergic receptors also seems unlikely in view of the lack of action of the α - and β -adrenergic agonists clonidine and isoproterenol. Furthermore, isoproterenol does not have its usual effect, an increase in intracellular cAMP levels, in isolated fish horizontal cells (O'Connor, Kropf and Dowling 1989).

If NE causes a release of dopamine from interplexiform cells and NE can act as an agonist at dopamine receptors, it would be expected that application of exogenous NE at sub-saturating concentrations would have a greater effect on HC receptive-field size in normal retinas than in retinas where the endogenous source of dopamine had been removed by 6-hydroxydopamine lesioning. Indeed, horizontal cell receptive-field size was more reduced in retinas treated with 30 μ M than 3 μ M NE (Fig. 9), yet 3 μ M NE caused a qualitatively similar change in horizontal cell receptive-field size in both control and 6-hydroxydopamine lesioned retinas (Fig. 7). It is therefore not clear why the removal of dopaminergic neurons by 6-hydroxydopamine lesioning did not reduce the effect of NE on horizontal cells. This result suggests that either 1) NE does not release endogenous dopamine and the observed NE-induced release of [3 H]-dopamine is a consequence of pre-loading, or 2) the endogenous dopamine released by NE does not reach the dopamine receptors on horizontal cells. Little is known about the function of the synaptic outputs of dopaminergic interplexiform cells that have been identified in the inner

fish retina (Yazulla and Zucker 1988). It is possible that the effects of any NE-induced dopamine release may be limited to the inner plexiform layer, and has no effect on horizontal cells. Unfortunately, these two possibilities could not be distinguished in the present experiments. Attempts to measure NE-evoked release of endogenous dopamine by HPLC were equivocal for technical reasons. Furthermore, our experiments did not address possible effects of NE-induced dopamine release in the inner retina.

The results of the present experiments on the effects of NE on the goldfish retina are summarized in Fig. 14. Our findings suggest that NE is taken up by a transport system in exchange for dopamine. It is possible that the normal function of such a transport mechanism is for re-uptake of released dopamine, and that the observed release of [³H]-dopamine is a consequence of pre-loading. On the other hand, the purpose of such a mechanism may be to release dopamine in response to increases in the extracellular concentration of NE, or some other phenylethylamine. It is not known if such a mechanism could operate in the absence of pre-loading with dopamine *in vivo*. It is also not known if such an exchange mechanism may act in the outer retina as depicted (Fig. 14), in the inner retina, or both. In addition to any effect NE may have on dopamine release, NE also appears to act as an agonist at horizontal cell D₁ dopamine receptors.

Figure 14 Schematic diagram summarizing effects of NE in the goldfish retina. Immunohistochemistry localized a putative norepinephrine (NE) releasing dopamine- β -hydroxylase immunoreactive amacrine cell type (DBH-AC) in the inner retina. Release experiments showed that NE caused [3 H]-DA release, but this release was not receptor mediated, suggesting activation of a DA exchange mechanism in dopaminergic interplexiform cells (DA-IPC). Such a release mechanism might reduce horizontal cell (HC) receptive-field size by acting at D₁ DA receptors *in vivo* (1). Electrophysiology experiments showed that NE reduced horizontal cell receptive-field size, but this effect was also present in 6-OHDA-lesioned retinas and blocked by haloperidol, suggesting that NE has a direct effect on HC DA receptors (2). OPL = outer plexiform layer; IPL = inner plexiform layer.



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7.0 Acknowledgements

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V. A new type of interplexiform cell
in the goldfish retina is immunoreactive
for PNMT

1.0 Abstract

A new cell type immunoreactive for phenylethanolamine N-methyltransferase, the synthesizing enzyme for epinephrine, has been identified in the goldfish retina. The somata of the immunoreactive cells were located in proximal inner nuclear layer and immunoreactive processes were located in both the inner and outer plexiform layers, suggesting that this cell may be an interplexiform cell. Confocal microscopy was used to establish that the putative adrenergic neurites in the outer plexiform layer were located between the somata of horizontal cells and photoreceptor cell synaptic terminals. Intracellular recordings from horizontal cells demonstrated that epinephrine had an effect on horizontal cells, but that the action of epinephrine was consistent with an effect on dopamine receptors, rather than adrenergic receptors. The function of the putative 'I3' interplexiform cell therefore remains unclear, possibly modulating horizontal cell function with a transmitter other than epinephrine or affecting photoreceptors or the retinal pigment epithelium rather than horizontal cells.

2.0 Introduction

The vertebrate retina is made up of five major classes of neurons. Photoreceptors, bipolar, and ganglion cells carry information vertically from outer to inner retina. Horizontal and amacrine cells provide lateral interactions where these cells synapse in the outer and inner plexiform layers, respectively. Interplexiform cells differ from these major cell types in that they provide an intrinsic centrifugal pathway from the inner retina back to the outer retina (Dowling 1987). Interplexiform cells were first identified in the teleost retina using the Falk-Hillarp method (Ehinger, Falck and Laties 1969) which causes certain monoamines to fluoresce. These dopaminergic interplexiform cells (Dowling and Ehinger 1978; Sarthy and Lam 1979; Yazulla and Zucker 1988) have been designated 'I1' retinal cells in the fish (Marc and Lam 1981). Subsequently, another glycine-containing interplexiform cell has been described (Marc and Liu 1984) and designated 'I2' (Marc and Lam 1981).

In the present study we provide immunohistochemical evidence supporting the existence of a third interplexiform cell in goldfish retina that is immunoreactive for phenylethanolamine N-methyltransferase (PNMT), the synthesizing enzyme for epinephrine. The potential target of transmitter released from the distal processes of interplexiform cells in the outer retina may be pigment epithelial cells, photoreceptors, horizontal cells, or bipolar

cells. The effect of epinephrine on one possible target of the putative adrenergic PNMT-IR interplexiform cell, horizontal cells, was also investigated.

3.0 Materials and methods

3.1 Immunocytochemistry

Goldfish (*Carassius auratus*) 6" - 8" in length were decapitated, pithed, the eyes enucleated and hemisected. Eyes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3 containing 1% sucrose, 0.2 mM CaCl_2 , for 1 h and then washed for 2 h in Rinse buffer (0.1 M sodium phosphate buffer at pH 7.3, 3% sucrose, 0.2 mM CaCl_2). Eyes were cryoprotected in 20% sucrose in Rinse buffer overnight, mounted in OCT compound, 10-15 μm frozen sections cut on a cryostat, sections picked up on 3-aminopropyltriethoxysilane - treated slides (APTEX, Sigma Chemical Co., St. Louis, MO), and dried for 2 h at room temperature. Slides were washed in phosphate-buffered saline to remove OCT compound. PNMT-immunoreactivity was localized by incubation in primary rabbit antiserum (PNMT from bovine adrenal; Eugene Tech International, Ramsey, NJ; 1:250) overnight followed by a secondary goat anti-rabbit antiserum conjugated to Texas Red (BioCan, Mississauga, ON; 1:100) for 4 h. The label was excited with a 543 nm HeNe laser for confocal microscopy using a Zeiss LSM-10.

3.2 High pressure liquid chromatography

Goldfish were decapitated and pithed and the retinas isolated in cold Ringer's solution. Four retinas for each

assay were homogenized in 200 μ l of 0.2N perchloric acid, immediately frozen on dry ice, and stored at -70°C until analysis. When ready for analysis, samples were thawed, centrifuged, extracted on alumina and applied to the HPLC column. Catecholamines were identified using electrochemical detection.

3.3 Intracellular recording

Goldfish were dark-adapted for 1 h, decapitated and pithed. Horizontal cell responses were measured using conventional intracellular voltage recording from isolated superfused retinas. The Ringer consisted of 110 mM NaCl, 2.5 mM KCl, 20 mM NaHCO_3 , 0.1 mM MgSO_4 , 0.7 mM CaCl_2 , 20 mM D-glucose, bubbled continuously with 95% O_2 5% CO_2 , pH 7.2, maintained at 20°C . All drugs were obtained from Research Biochemicals Inc. (Natick, MA) and applied to the retina after being dissolved in the superfusate. Ascorbic acid (0.1 mM) was added to the Ringer containing epinephrine, as an antioxidant. The recording system has been described in detail elsewhere (Baldrige and Ball 1991). Horizontal cell receptive-field size, which is related to the extent of gap junction coupling between horizontal cells, was measured by comparing the responses of H2 horizontal cells to slit (7mm x 250 μ m, $-3.0 \log I_0$) and annulus (inner diameter: 2.5mm, outer diameter: 6.5 mm, $-3.5 \log I_0$) white light stimuli ($I_0=10^{14}$ photons $\text{cm}^{-2} \text{sec}^{-1}$ at 500nm). The effect of each treatment was

done on at least three cells and similar results were obtained in each case.

4.0 Results

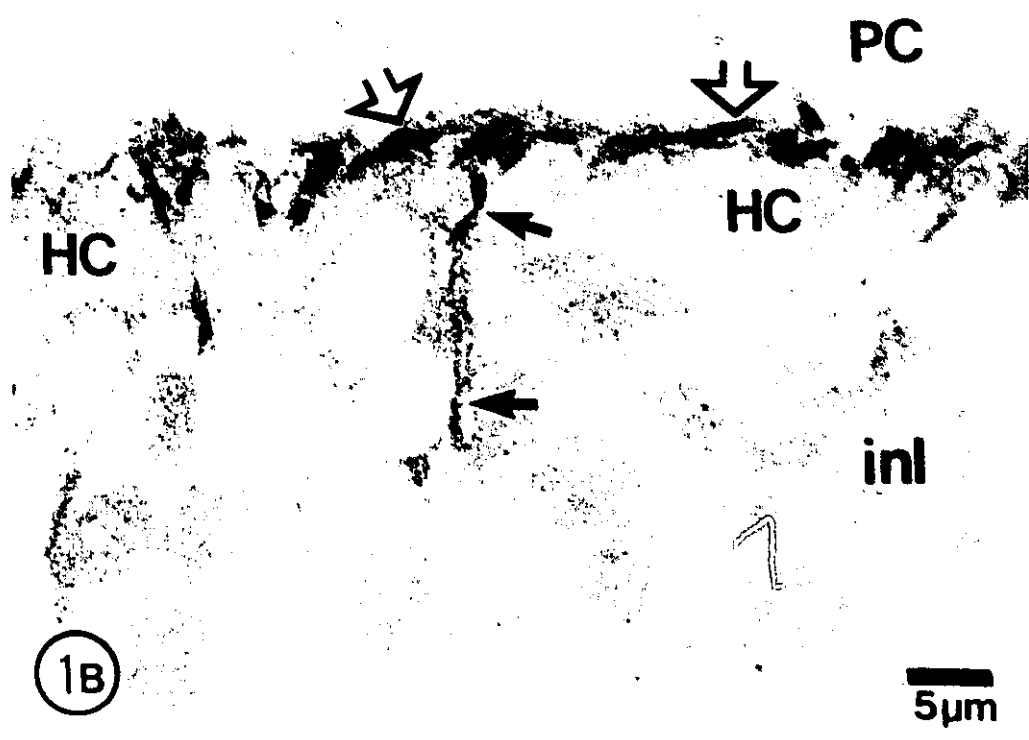
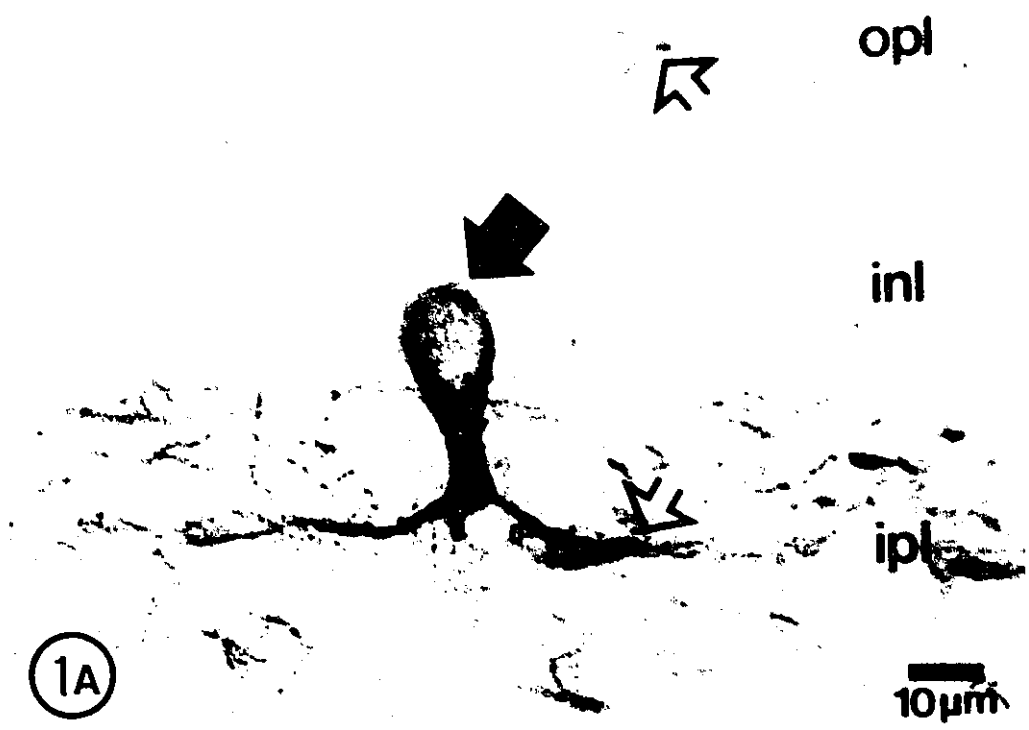
4.1 Immunocytochemistry

PNMT-immunocytochemistry labelled a population of large round cells measuring $12\ \mu\text{m}$ in diameter, located in the first tier of amacrine cells in the inner nuclear layer (Fig. 1A). The density of cells was estimated at $120/\text{mm}^2$. Less than 1% of the total number of PNMT-IR somata were located in the ganglion cell layer. The majority of cells had a $3\ \mu\text{m}$ -thick process extending to the middle of the inner plexiform layer. PNMT-immunoreactive neurites were located in each substrata of the IPL. A plexus of PNMT-immunoreactive fibres was also located in the outer plexiform layer, distal to horizontal cell somata (Fig. 1B). Processes were observed extending through the inner nuclear layer between the inner plexiform and outer plexiform layers (Fig. 1B), but never directly from PNMT-immunoreactive somata. Preabsorbing the primary antiserum with PNMT (Sigma #8924; 5 mg/ml) overnight abolished all staining.

4.2 High pressure liquid chromatography

In addition to dopamine and norepinephrine, epinephrine was identified in the homogenate of four retinas (Fig. 2B). The peaks of the chromatogram from the retinas were identified by comparison to the chromatogram of a series of standards

Figure 1 Laser scanning micrographs showing phenylethanolamine N-methyltransferase (PNMT)-like immunoreactivity of a putative epinephrine-containing interplexiform cell and processes from goldfish retina. (A), Low magnification composite overlay of ten $0.25\mu\text{m}$ -thick confocal sections showing a PNMT-immunoreactive (IR) soma located in the proximal INL. Most PNMT-IR cells (arrow) had a thick primary process extending to the middle of the IPL before extending laterally. PNMT-IR interplexiform cell processes were located in both the inner plexiform layer (IPL) and outer plexiform layer (OPL) (open arrows). (B), High magnification $0.25\mu\text{m}$ -thick confocal section showing the PNMT-IR plexus in the OPL (open arrows). PNMT-IR terminals measuring $<1\mu\text{m}$ were located in the OPL, between horizontal cell somata (HC) and photoreceptors (PC) but did not extend into the photoreceptor synaptic terminal itself. Ascending PNMT-IR processes were observed in the INL (small arrows) extending between the IPL and OPL.



(norepinephrine, epinephrine, internal standard and dopamine) (Fig. 2A). The amount of epinephrine per retina was estimated to be <1 pg.

4.3 Horizontal cell recordings

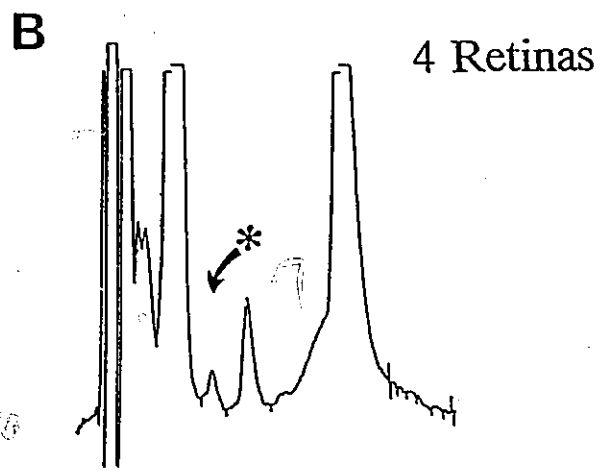
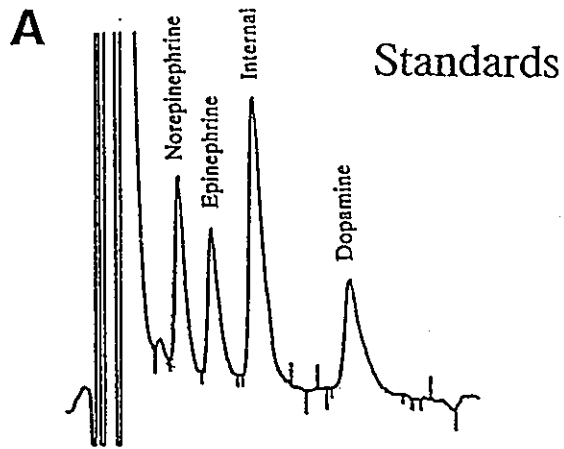
The typical dark resting membrane potential of the horizontal cells recorded from was -25 mV. Epinephrine (3 μ M) usually depolarized horizontal cells and always reduced their receptive-field size as indicated by an increase in the response to slit stimuli and a decrease in the response to annulus stimuli (Fig. 3A). However, these effects were not blocked by either the α - or β -adrenergic receptor antagonists, phentolamine (50 μ M) (not shown) or propranolol (50 μ M) (Fig. 3B), respectively. However, the effect of epinephrine was blocked by the dopamine D₁ receptor antagonist SCH-23390 (50 μ M) (Fig. 3C).

5.0 Discussion

Using an antibody directed against the synthesizing enzyme for epinephrine, phenylethanolamine N-methyltransferase (PNMT), we have identified a population of presumed interplexiform cells (IPCs) in the goldfish retina. The PNMT-immunoreactive somata did not resemble previously described 'I1' dopaminergic or 'I2' glycinergic IPCs in size, shape, or location. The outer PNMT-immunoreactive plexus also differed from the plexus of dopaminergic 'I1' IPCs in that PNMT-immunoreactive processes were restricted to the outer

Figure 2 High pressure liquid chromatograph of goldfish retinal homogenate. (A) Chromatograph of standards: norepinephrine, epinephrine, internal standard and dopamine. (B) Chromatograph of homogenate from four retinas. The epinephrine peak is indicated by asterisk and arrow. Other peaks correspond to compounds in same position as in the Standards chromatogram (A).

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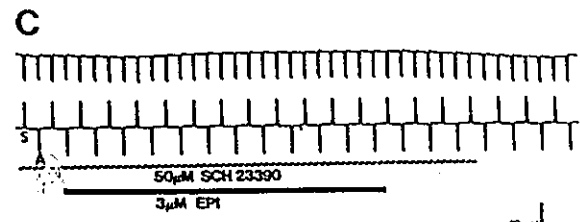
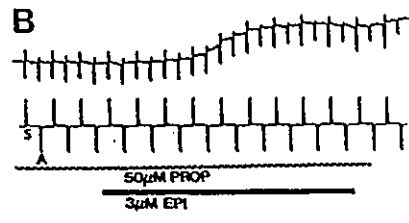
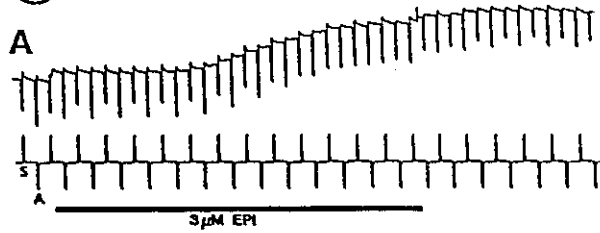


plexiform layer rather than enveloping horizontal cell somata. Furthermore, additional double-labelling experiments using rabbit anti-PNMT and sheep anti-tyrosine hydroxylase antisera demonstrated that both the somata and the outer plexiform layer plexus of these two cell types were distinct. We presume that the OPL PNMT-immunoreactive plexus therefore represents the terminal arborization of a new class of IPC. However, because some PNMT-immunoreactive somata were localized in the ganglion cell layer it seems unlikely that all PNMT-immunoreactive cells contribute to the outer plexiform layer plexus. The localization of the synthesizing enzyme for epinephrine in the goldfish retina suggests that this transmitter must play a role in retinal processing.

Other investigators have reported the presence of epinephrine in the fish retina, at levels several orders of magnitude less than dopamine (Dearry and Burnside 1989), or find no epinephrine at all (O'Connor, Kropf and Dowling 1989). High pressure liquid chromatography analysis of goldfish retina confirmed the presence of epinephrine at <1 pg/retina. Although epinephrine has also been identified in rat and bovine retinas (Wyse and Lorscheider 1981; Osborne and Nesselhut 1983), PNMT-immunoreactive neurons in the rat and ferret retina have been localized to amacrine cells and horizontal cells (Hadjiconstantinou et al. 1984; Keyser et al. 1987) rather than IPCs. The rat PNMT-immunoreactive neurons have been reported by some to be distinct from tyrosine

Figure 3 Effect of epinephrine (EPI) on horizontal cell (HC) receptive-field size in the goldfish retina. The dark-resting membrane potential of horizontal cells was -25 mV. (A), Application of 3 μ m EPI depolarized the H2 HC membrane potential, increased the response to slit stimuli and decreased the response to annulus stimuli. These changes are indicative of a decrease in HC receptive-field size (Piccolino, Neyton and Gerschenfeld 1984; Dong and McReynolds 1991). (B), The effect of 3 μ m EPI was not blocked by the β -adrenergic antagonist propranolol (PROP) (50 μ m). (C), The effect of 3 μ m EPI was blocked by the dopamine D₁ antagonist SCH-23390 (50 μ m).

3



10 mV
10 sec

hydroxylase-immunoreactive neurons (Hadjiconstantinou et al. 1984; Hammang, Bohn and Messing 1992; Nguyen-Legros et al. 1985a) but others suggest the immunoreactivity is colocalized (Park et al. 1986).

The finding that the PNMT-immunoreactive cells we localized in the goldfish retina were not also tyrosine hydroxylase-immunoreactive raises the question of how epinephrine could be synthesized in the absence of other catecholamine synthesizing enzymes. In addition to the absence of PNMT- and tyrosine hydroxylase-immunoreactive colocalization in rat and ferret retina (Hadjiconstantinou et al. 1984; Keyser et al. 1987; Hammang, Bohn and Messing 1992) there are examples of other neurons in the central nervous system that do not show such colocalization (Anderson and Howe 1988; Ross et al. 1984). It has been suggested that the PNMT-immunoreactive neurons might synthesize epinephrine from some other substrate using an alternate synthetic pathway (Nguyen-Legros et al. 1985a). It is also possible that the PNMT-immunoreactive neurons we localized are not the source of the epinephrine we identified in the retina. If PNMT-immunoreactive IPCs do not contain epinephrine, which neurotransmitter they contain and why such cells continue to express PNMT is unclear.

The presence of PNMT-immunoreactive neurites in the outer plexiform layer suggest that horizontal cells or photoreceptors may receive innervation from these putative

adrenergic terminals. To test the effect of epinephrine on one of these potential targets, horizontal cells, intracellular recordings from horizontal cells were made in isolated superfused goldfish retinas. We found that as little as 3 μ M epinephrine depolarized horizontal cells and reduced their receptive-field size. However, the effects of epinephrine were not blocked by either α - or β -adrenergic receptor antagonists, but were blocked by the dopamine D₁ receptor antagonist SCH-23390. This suggests that exogenous epinephrine affects horizontal cells by inducing the release of endogenous dopamine from 'I1' IPCs or by the direct action of epinephrine on horizontal cell D₁ dopamine receptors (Seeman 1981; Vanderhayden et al. 1986). In either case, these results provide evidence that epinephrine does not act on horizontal cells through an adrenergic receptor mechanism. The absence of β -adrenergic receptors on teleost horizontal cells is consistent with the report that a β -receptor agonist does not increase intracellular cAMP in isolated teleost horizontal cells (O'Connor, Kropf and Dowling 1989). Another possible target of adrenergic innervation from IPCs are photoreceptors or the retinal pigment epithelium. The latter has been reported to possess α -adrenergic receptors that may regulate pigment dispersion (Dearry and Burnside 1988).

6.0 Conclusions

A new type of interplexiform cell has been identified in the goldfish retina which is immunoreactive for PNMT, the

synthesizing enzyme for epinephrine. Epinephrine, the putative transmitter of the interplexiform cell, depolarized and reduced the receptive-field size of horizontal cells, possible targets of the outer plexiform layer plexus formed by some PNMT-immunoreactive cells. However, pharmacological experiments demonstrated that the observed effect of epinephrine on horizontal cells was mediated by dopamine receptors rather than adrenergic receptors. Unless epinephrine affects horizontal cells in a non-traditional manner by acting directly on dopamine receptors (Seeman 1981; Vanderhayden et al. 1986), this finding suggests that horizontal cells are unlikely to be the target of the putative epinephrine-containing PNMT-immunoreactive interplexiform cells. Although the neurotransmitter and target of the PNMT-immunoreactive IPCs remains to be elucidated, the present study has identified a new type of IPC in the teleost retina. The nomenclature established by Marc and Lam (1981) identifies the dopamine-containing IPC as 'I1' and the glycinergic IPC as 'I2'. We therefore propose that the PNMT-immunoreactive IPC described here be given the designation 'I3'.

7.0 Acknowledgements

This work was supported by a grant to A.K.B. by the Medical Research Council of Canada. W.H.B. is supported by a Medical Research Council of Canada Studentship.

VI. The possible role of nitric oxide in the
light-induced reduction of
horizontal cell receptive-field size

1.0 Summary and significance to thesis

This work was presented at the 1993 Association for Research in Vision and Ophthalmology annual meeting (Baldrige, Jamieson and Ball 1993).

This chapter presents evidence that the free radical gas nitric oxide may be involved in the light-induced reduction of horizontal cell receptive-field size (light-adaptation) described in Chapters II and III. This was demonstrated by showing that two inhibitors of nitric oxide synthase reduce the effects of background illumination on horizontal cell receptive-field size, an effect that can be partially reversed by subsequent treatment with L-arginine. Although physiological studies by others have also indicated a possible role of nitric oxide in horizontal cells (DeVries and Schwartz 1989; Miyachi, Murakami, and Nakaki 1990), our studies were prompted in large part by our observation that fish horizontal cells are nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH)-positive (a marker of nitric oxide synthase) and nitric oxide synthase-immunoreactive (Fig. 1).

The results of this study suggest that nitric oxide is involved in mediating the effects of background illumination

on horizontal cell receptive-field size. In Chapter III it was demonstrated that background illumination has two effects on horizontal cell receptive-field size in light-sensitized retinas. Background illumination 1) caused a rapid decrease in receptive-field size only while the background illumination was on, and 2) later caused a decrease in receptive-field size that persisted even after background illumination was turned off. The results presented in this study suggests that nitric oxide acts as a modulator of the mechanism that rapidly reduces horizontal cell receptive-field size in the presence of background illumination. Nitric oxide, produced by background illumination, permits this mechanism to respond quickly to changes in light or changes in horizontal cell membrane potential that accompany light. This means that NO would be necessary, but not sufficient, to produce a change in horizontal cell receptive-field size during background illumination. It is unlikely that nitric oxide alone could directly mediate the reduction in horizontal cell receptive-field size during background illumination because of the slow kinetics of the likely second messenger system associated with nitric oxide, guanosine 3',5'-monophosphate (cyclic GMP), produced by soluble guanylate cyclase. However, it is possible that nitric oxide is involved directly in the persistent decrease in receptive-field size of horizontal cells after light-adaptation, an effect that may be a consequence of higher retinal levels of nitric oxide than

necessary to trigger the mechanism that transiently reduces horizontal cell receptive-field size during background illumination. It is suggested, therefore, that this sustained effect is independent of the nitric oxide-activated voltage-dependent mechanism that operates during background illumination.

2.0 Abstract

Goldfish retinal horizontal cells were shown to be nicotinamide adenine dinucleotide phosphate-diaphorase positive and nitric oxide synthase-immunoreactive. The possible role of nitric oxide in the regulation of horizontal cell receptive-field size was investigated by studying the effect of two inhibitors of nitric oxide synthase, N_G -nitro-L-arginine or N^G -nitro-L-arginine methyl ester, on the light-dependent reduction of receptive-field size in light-sensitized goldfish retinas. Application of background illumination to light-sensitized goldfish retinas initially caused a reduction in receptive-field size during background illumination and later a reduction in receptive-field size that persisted even after the background light was turned off, a condition leading to light-adaptation. Superfusion of isolated goldfish retinas with either nitric oxide synthase inhibitor reduced both the effect of background illumination on horizontal cell receptive-field size during and after application of the background adapting light. The effect of the inhibitors during background illumination could be partially reversed by subsequent superfusion of inhibitor-treated retinas with Ringer containing L-arginine. However, recovery of the persistent change of horizontal cell receptive-field size after background illumination was not observed after L-arginine treatment. This may be due to either an incomplete recovery of the nitric oxide system in

the retina after inhibitor treatment or insufficient light exposure after inhibitor treatment. *In toto*, these results suggest that nitric oxide may play a role in the reduction of horizontal cell receptive-field size by background illumination.

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3.0 Introduction

Horizontal cells are second-order neurons of the vertebrate retina that are involved in the generation of the receptive-field surround of bipolar cells and ganglion cells (Werblin and Dowling 1969; Werblin 1970; Naka and Nye 1971; Naka and Witkovsky 1972; Naka 1977; Toyoda and Tonosaki 1978; Toyoda and Kujiraoka 1982; Sakuranga and Naka 1985). A key feature of horizontal cells is that they are coupled extensively by gap junctions (Yamada and Ishikawa 1965; Naka and Rushton 1967; Stell 1967; Witkovsky and Dowling 1969; Kaneko and Stuart 1980, 1984) and, as a result, the receptive-field size of horizontal cells is larger than the size of their dendritic field.

The receptive-field size of horizontal cells is not static but changes according to the adaptation state of the retina. Horizontal cell responsiveness and receptive-field size is decreased in the dark-adapted retina (Mangel and Dowling 1985). This effect is thought to be due to the action of dopamine, the only known modulator of horizontal cell receptive-field size (Negishi and Drujan 1979a, 1979b; Negishi, Teranishi and Kato 1983; Teranishi, Negishi and Kato 1983; Mangel and Dowling 1985). When dark-adapted retinas are exposed to light the responsiveness and receptive-field size of horizontal cells increases. This process has been termed light-sensitization (Tornqvist, Yang and Dowling 1988; Yang, Tornqvist and Dowling 1988a, 1988b; Mangel et al. 1993) and is

believed to be due to decreased dopamine release. When light-sensitized retinas are exposed to additional light the receptive-field size of horizontal cells decreases (Shigematsu and Yamada 1988) but this effect has been shown not to depend on the presence of dopamine (Baldrige and Ball 1991; Umino, Lee and Dowling 1991). The light-induced reduction of horizontal cell receptive-field size in light-sensitized retinas has been termed light-adaptation (see Chapter III). The mechanism by which light reduces the receptive-field size of light-sensitized horizontal cells is unknown.

Nitric oxide (NO) is a free radical gas that has recently been identified as a diffusible mediator of cell-to-cell communication in a variety of tissues including the nervous system (see Moncada, Palmer and Higgs 1989; Bredt and Snyder 1992 for review). NO is produced by the conversion of L-arginine (L-ARG) to L-citrulline catalyzed by the enzyme nitric oxide synthase (NOS) (Bredt and Snyder 1990). Unlike a conventional neurotransmitter, NO is not contained within synaptic vesicles but is found in the cytosol (Schmidt et al. 1989; Llewellyn-Smith et al. 1992). Therefore, for NO to act as a transmitter it would have to diffuse across membranes and through the extracellular space to reach its target. NO is a potent activator of soluble guanylate cyclase (Katsuki et al. 1977) and has been shown to increase the levels of cyclic GMP in a variety of neurons (Miki, Kawabe and Kuriyama 1977; Yoshikawa and Kuriyama 1980; Förstermann et al. 1990; Reiser

1990; Kiedrowski, Costa and Wroblewski 1992).

The first indication that NO might be involved in the modulation of retinal horizontal cell activity came from the observation by DeVries and Schwartz (1989) that cyclic GMP, when injected into cultured catfish horizontal cells, decreased electrical coupling between isolated pairs of these cells. Later, Miyachi and Murakami (1991) showed that intracellular injection of cyclic GMP into carp and turtle horizontal cells in isolated retinas increased the input resistance of these cells, an effect consistent with a decrease in coupling. An association between cyclic GMP and NO in horizontal cells arose from studies showing that intracellular application of sodium nitroprusside, a NO donator, reduced catfish and turtle horizontal cell junctional conductance (DeVries and Schwartz 1989; Miyachi, Murakami and Nakaki 1990). Furthermore, intracellular injections of L-ARG, the substrate of NOS that results in NO production, increased turtle horizontal cell input resistance, an effect that could be blocked by N-methyl-L-arginine (Miyachi, Murakami and Nakaki 1990) a specific inhibitor of NOS (Knowles et al. 1990).

In the present work we have investigated the possibility that NO might be involved in regulating horizontal cell receptive-field size changes during application of background illumination to light-sensitized retinas. First, it was determined if horizontal cells contain NOS by determining if

they were nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH)-diaphorase positive or NOS-immunoreactive. Next, the effect of two selective blockers of NOS on the light-induced reduction of horizontal cell receptive-field size were studied in the isolated superfused goldfish retina.

4.0 Materials and Methods

4.1 Localization of cells producing nitric oxide

Goldfish eyes were enucleated, hemisected and the lens removed. The resulting eyecups were immersed in fixative consisting of 4% paraformaldehyde in 0.1M sodium phosphate buffer pH 7.3 containing 2% sucrose and 0.2 mM CaCl₂ for 1-2 hrs. Eyecups were washed in Rinse Buffer consisting of 0.1M sodium phosphate buffer, 3% sucrose, and 0.2 mM CaCl₂ for 2-8 hrs. During this time they were trimmed of excess connective tissue adhering to the sclera, and the vitreous was wicked away with filter paper. Eyecups were immersed in 25% sucrose (w/V) in Rinse Buffer for 4 hrs and infiltrated with 50% OCT compound 50% high sucrose Rinse Buffer for 1-4 hrs on a rotator. Eyecups were frozen in OCT compound on metal stubs and sectioned at 5, 12, or 20 μ m on a cryostat. Sections were thaw-mounted on APTEX- (Sigma #A3648, St. Louis, Mo.) treated slides.

For histology, 5 μ m-thick sections were rinsed in phosphate buffered saline (PBS) to remove OCT compound and immersed in 1% aqueous toluidine blue for 1 min. Slides were rinsed in PBS and coverslipped in glycerol.

For NADPH-diaphorase histochemistry, 20 μm -thick sections were rinsed in PBS. Slides were positioned horizontally in a humid chamber and the sections flooded with 100 μl of a solution consisting of 0.1 M TRIS-HCl buffer pH 8.0, 1 mM β -NADPH (Sigma kit #201-201), 0.5 mM Nitro blue tetrazolium (Sigma # N5514), 0.25% Triton X-100, and 1% DMSO) (Mizukawa et al. 1988). Slides were incubated at room temperature for 1, 2, or 4 hrs. The slides were then rinsed in PBS and coverslipped in glycerol.

For NOS immunohistochemistry, 12 μm -thick sections were rinsed in PBS. Sections were incubated in 100 μl Rinse Buffer containing 1% normal goat serum, 1% DMSO, and 0.05% Triton-X 100 for 1 hr at room temperature in a humid chamber. Sections were then incubated in a well-characterized antisera raised in rabbit against NOS-I isolated from rat cerebellum (rabbit anti-NOS polyclonal #6761-7; Abbott Laboratories, North Chicago, Ill.; 1:500, 1% NGS, 1% DMSO, 0.05% Triton-X 100) (Dun et al. 1992; Matsumoto et al. 1992; Schmidt et al. 1992) for 8 hrs. Sections were washed in PBS and the primary antibody was revealed using the *ELite* ABC technique (VectaStain PK-6101, Dimension Laboratories, Mississauga, ON) with diamino-benzidine (SigmaFast D4418) as the chromogen. Slides were washed in PBS and coverslipped in VectaShield (Vector Labs #H-1000).

Sections were viewed in transmitted mode on a Zeiss LSM-10 and digitized images printed using a Mitsubishi CP-110U.

4.2 Intracellular recordings

All procedures and analysis, including retina preparation, intracellular recording technique, background illumination, receptive-field size measurement, were as described in Chapter III. All experiments were performed on light-sensitized retinas. The retina was considered to be light-sensitized because their responses to full-field illumination at $-3.0 \log I_0$ produced responses of more than 10 mV. In some cases, the retina was exposed to background illumination prior to drug treatment to be certain that the retina was in a light-sensitized state.

4.3 Treatments

Prior to application background illumination, the retina was treated for 20-30 min with an NOS blocker, either 100 μM N_ω -nitro-L-arginine (L-NOLA; Sigma) or 100 μM N^ϵ -nitro-L-arginine methyl ester (L-NAME; Calbiochem, La Jolla, CA) dissolved in the superfusate Ringer. Both of these inhibitors block NO production by substituting the normal substrate for NOS, L-arginine, with L-arginine analogues that can bind competitively to NOS but do not result in the conversion to L-citrulline that produces NO (Knowles et al. 1990). The responses to slit and full-field stimuli (see Chapter III) were measured during and after the application of background illumination in the presence of these NOS blockers. In some retinas an attempt was made to reverse the effects of the blockers by superfusing the retina with 100 μM L-arginine (L-

ARG; Sigma) for 15-20 min to replace the L-arginine analogues with the normal substrate of NOS.

Because the isolated goldfish retina represents a < 1 mm thick "natural slice" of neuronal tissue (Rowe 1987) the concentration of the NOS inhibitors and L-ARG in the superfusate were similar to those that have been shown to block long term potentiation (LTP) in the rat hippocampal slice preparation (Bohme et al. 1991).

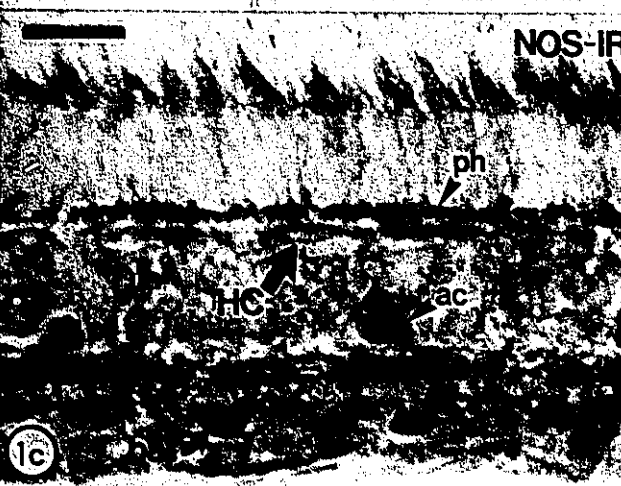
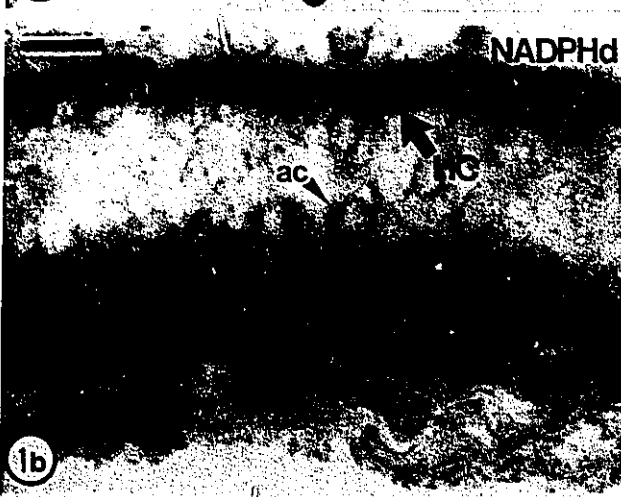
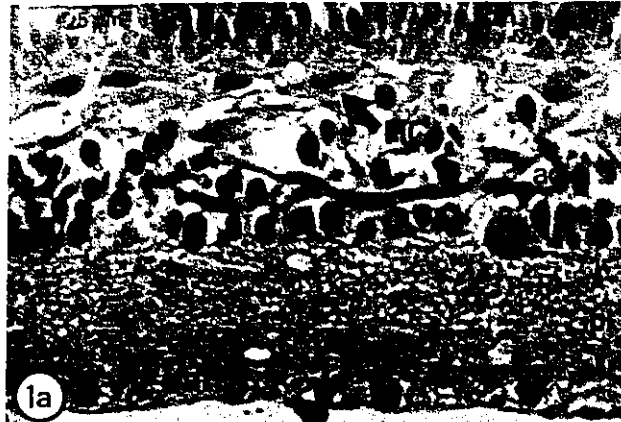
To compare the effects of background illumination of drug treated retinas with control retinas, the intensity and duration of the background illumination in this study was the same as previously used in studies of the effects of background illumination on light-sensitized retinas (Chapter III). All recording came from H2 horizontal cells.

5.0 Results

5.1 *Localization of cells producing NO*

Retinal neurons and their processes that were either NADPH-diaphorase positive or NOS-immunoreactive were identified by comparison to a section stained with toluidine blue (Fig. 1a). NADPH-diaphorase staining was localized to horizontal cells, amacrine cells and bipolar cells (Fig. 1b). A similar distribution of NOS-immunoreactivity was observed (Fig. 1c).

Figure 1 Micrographs showing putative sites of NO synthesis in the goldfish retina. The retinal location of NADPH-diaphorase staining and nitric oxide synthase-immunoreactivity was identified in toluidine blue-stained sections (Fig. 1a; ph = photoreceptors; HC = horizontal cell layer; ac = amacrine cell layer; bat = bipolar cell axon terminal; ip1 = inner plexiform layer). NADPH-diaphorase staining, which has been associated with NOS activity was localized to horizontal cells, amacrine cells and bipolar cells in 20 μm -thick sections (Fig. 1b). A similar distribution was seen in 12 μm sections incubated in rabbit anti-NOS and visualized using the ABC technique (Fig. 1c). Dense staining was observed in photoreceptor terminals (Fig. 1c, ph) and the axon terminals of ON bipolar cells (Fig. 1c, bat). In addition, staining was observed in horizontal cells (Fig. 1c, HC) and a sub-type of amacrine cell (Fig. 1c, ac). bar = 25 μm .



5.2 NOS inhibitors block the effect of light-adaptation on horizontal cells

Application of background illumination to a light-sensitized retina reduces the response of horizontal cells to full-field stimuli compared to the response to slit stimuli (Fig. 2a) indicating a decrease in horizontal cell receptive-field size (Piccolino, Neyton and Gerschenfeld 1984; Mangel and Dowling 1987). This effect first occurs only in the presence of background illumination but after additional background illumination, the increase in slit response relative to full-field response persists even after the background light is turned off. A persistent reduction of horizontal cell receptive-field size during background illumination was never observed in the absence of a change during the application of background illumination.

After treatment with 100 μ M N_G-nitro-L-arginine (L-NOLA) for 20-30 min the effect of background illumination on horizontal cell receptive-field size was reduced in 10/10 cells studied (Fig. 2b, 3). Treatment with L-NOLA did not alter horizontal cell dark resting membrane potential. When the retina was then superfused with Ringer containing 100 μ M L-ARG for 15-20 min, and another horizontal cell impaled, background illumination caused a reduction in horizontal cell receptive-field size during background illumination (Fig. 4, 5). The effect of background illumination on horizontal cell receptive-field size was not as marked in retinas pretreated

Figure 2 Effect of background illumination on a horizontal cell from a light-sensitized retina before and after treatment with 100 μM N ω -nitro-L-arginine. Prior to background illumination the intensity of the slit (S) was adjusted to be similar or less than the response to full-field (FF) stimuli. (A) In control retina, the response to full-field stimuli was decreased relative to slit stimuli during and after background illumination. (B) After treatment with 100 μM N ω -nitro-L-arginine the response to full-field stimuli remained greater than the response to the slit during and after background illumination. Slit intensity $-2.4 \log I_0$, full-field intensity $-3.7 \log I_0$, background intensity $-2.5 \log I_0$. Stimulus time length was 350 msec and ordinate indicates membrane potential in mV.

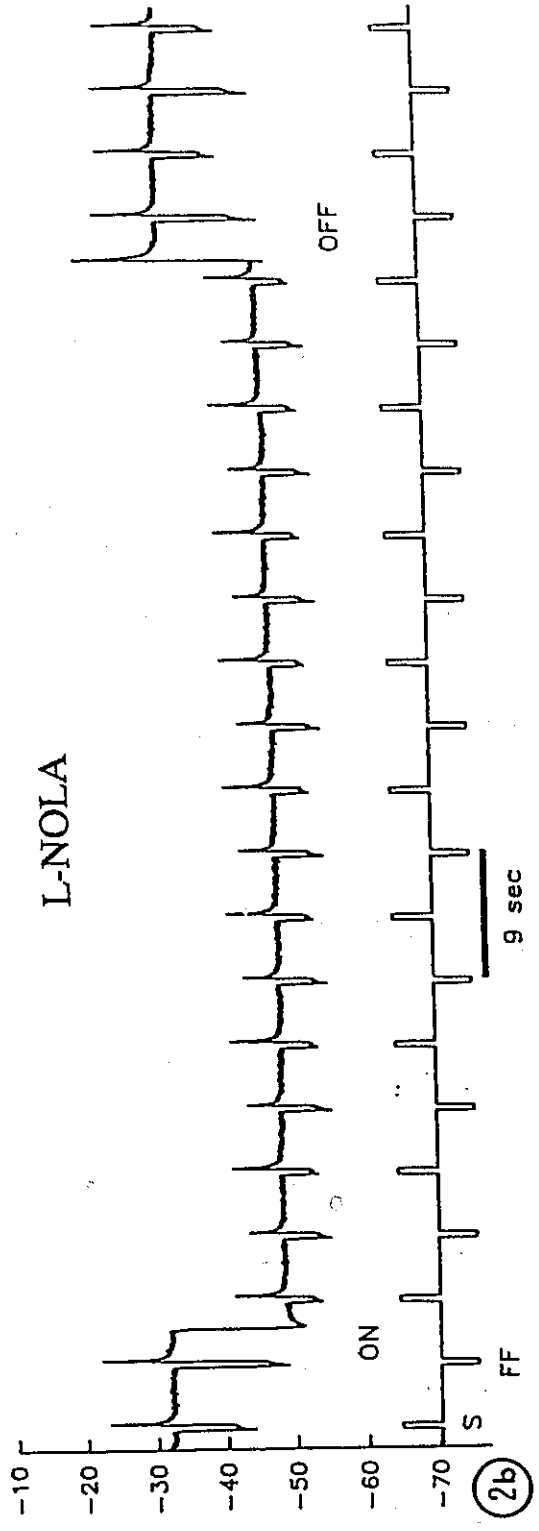
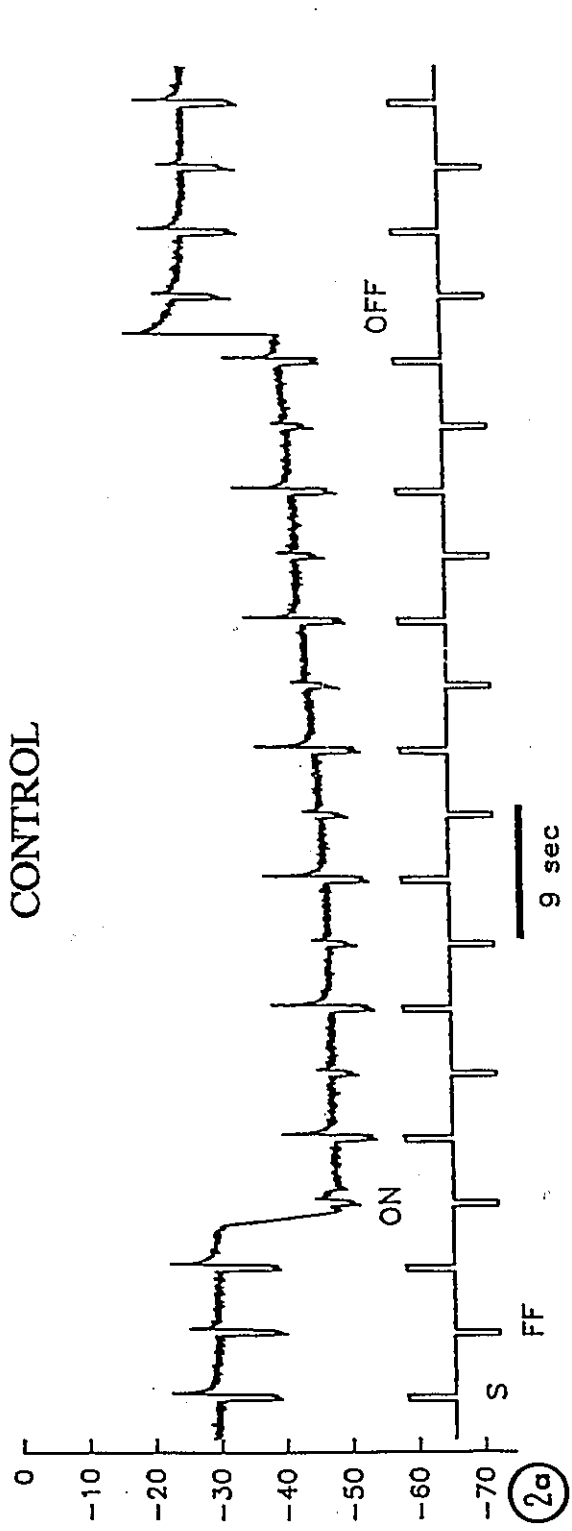


Figure 3 Details of the response waveform of the horizontal cell depicted in Fig. 2B. (A) Hyperpolarizing transients at light onset were noted prior to background illumination (filled arrow) as were depolarizing transients at light off (open arrow) indicating that the retina was light-sensitized. The ratio of slit/full-field response was 0.72. (B) In the presence of background illumination the horizontal cell was hyperpolarized, the response to stimuli decreased but the response to full-field stimuli was still greater than the response to slit stimuli. The slit/full-field response ratio increased only by 0.08 to 0.80. In this example the hyperpolarizing transient at light onset and the depolarizing transient at light off were reduced in the presence of background light but this was not typical of all cells studied (see Fig. 7). (C) After the background light was extinguished the response to full-field stimuli was still greater than the response to slit stimuli with a slit/full-field response ratio of 0.63. No light-sensitization was apparent because the response to full-field stimuli was less after background light than before. The hyperpolarizing transient at light onset was enhanced in the response to full-field stimuli after background illumination. There was a slight reduction of the depolarizing transient at light off after background illumination. Stimulus time length, intensities and ordinate details are identical to Fig. 2B.

L-NOLA

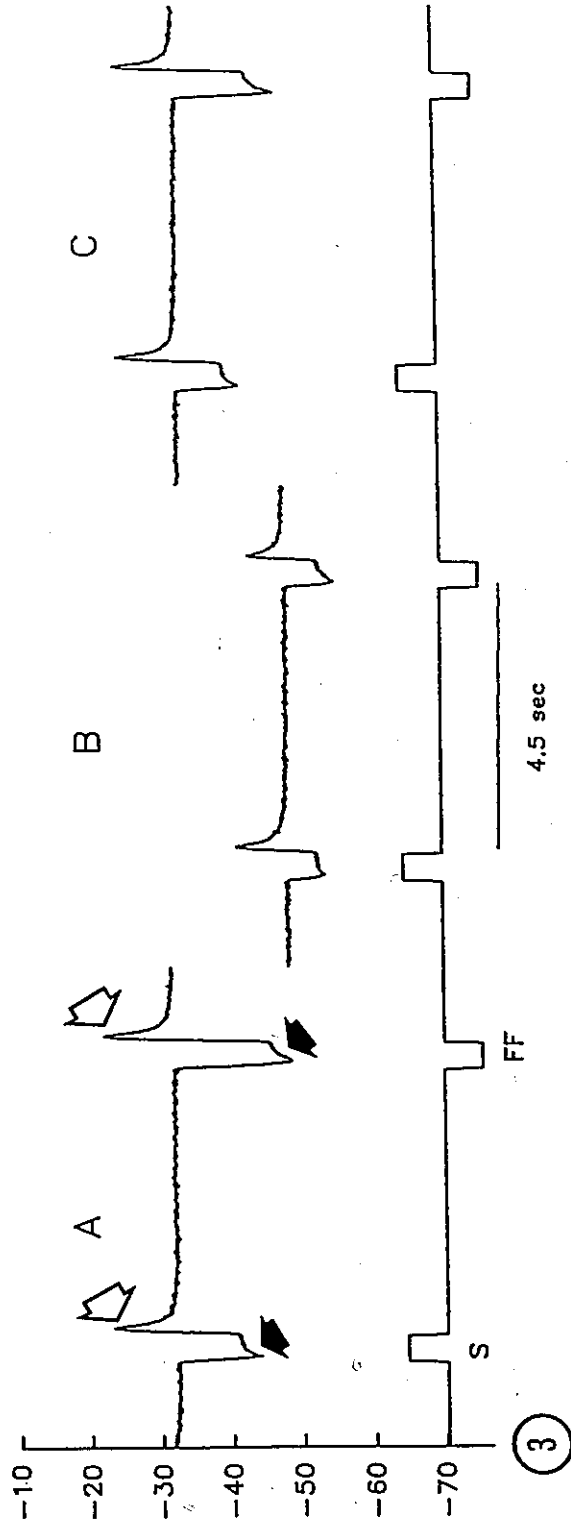


Figure 4 L-arginine partially reversed the effect of Nω-nitro-L-arginine on horizontal cell light-adaptation. This cell was from the same retina as in Fig. 2B but in a horizontal cell impaled after treatment with 100 μM L-arginine for 20 min. Prior to background illumination the response to full-field stimuli (FF) was somewhat greater than the response to slit (S) stimuli. During application of background light the response to full-field stimuli became less relative to the response to slit stimuli. After the background light was turned off the response to full-field stimuli was again greater than the response to slit stimuli. Slit intensity - 2.4 log I₀, full-field intensity -3.7 log I₀, background intensity -2.5 log I₀. Stimulus time length was 350 msec and ordinate indicates membrane potential in mV.

L-ARG RECOVERY FROM NOLA

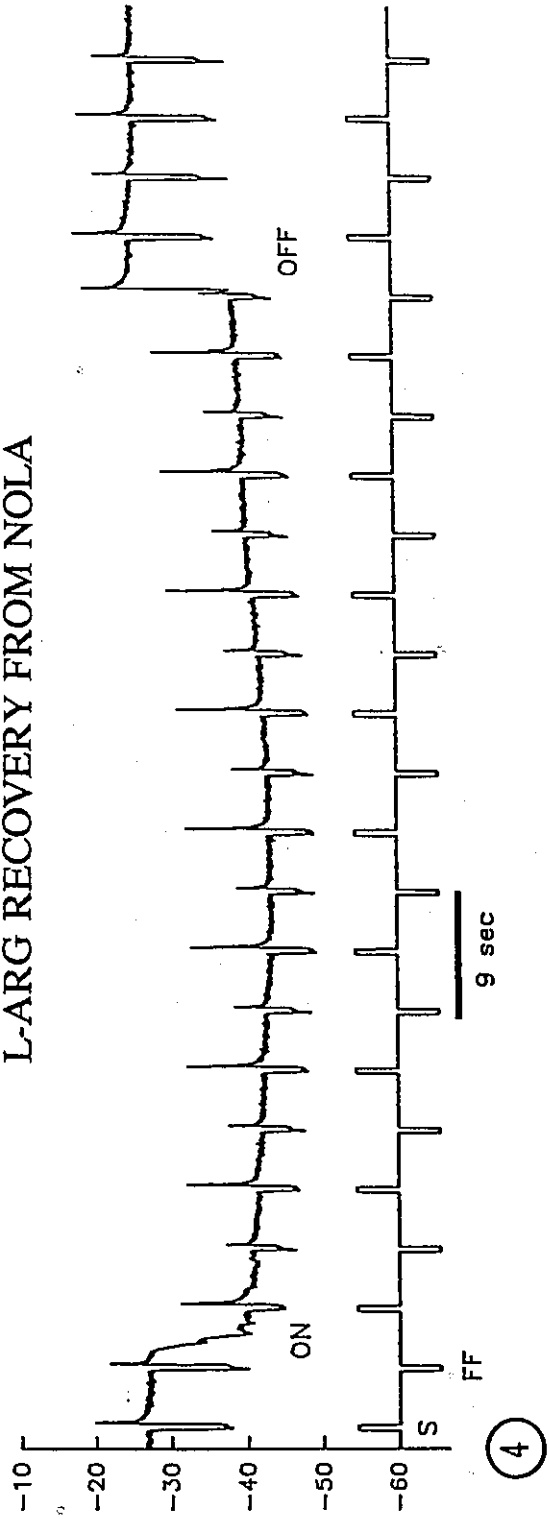
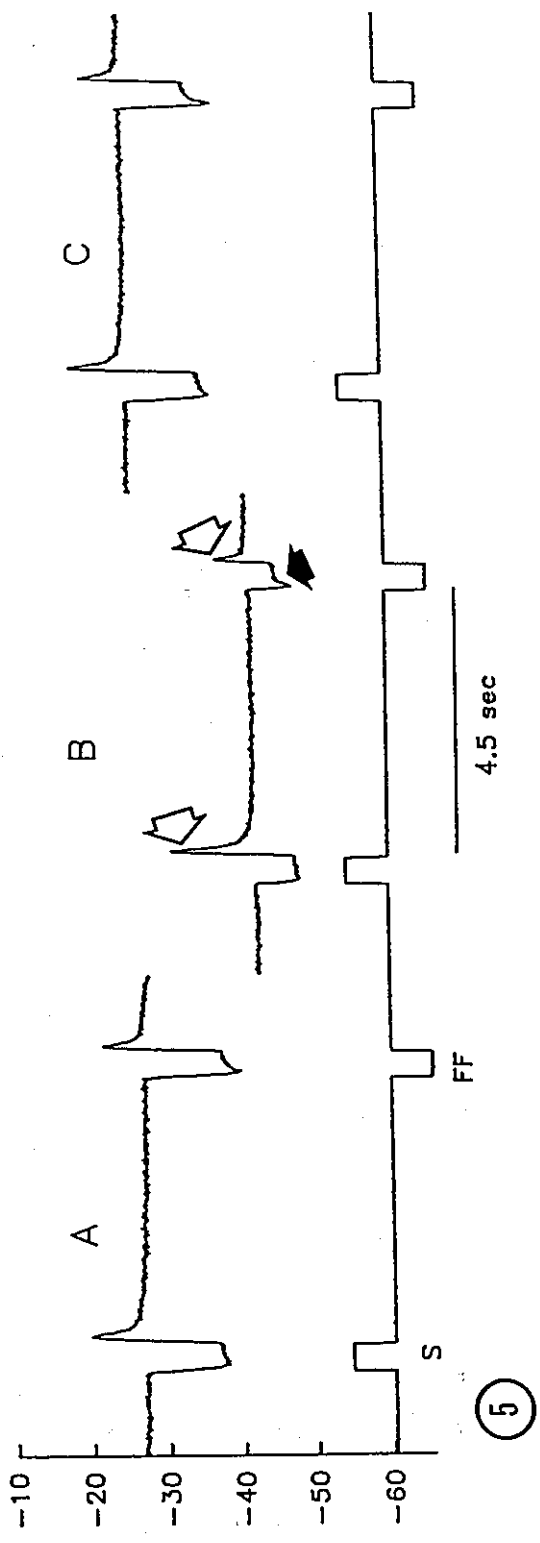


Figure 5 Details of the response waveform of the horizontal cell depicted in Fig. 4. (A) Prior to background illumination the response to full-field stimuli was greater than the response to slit stimuli. The slit/full-field response ratio was 0.87. (B) During presentation of background light the response to full-field stimuli was decreased relative to the response to the slit. The slit/full-field response ratio increased by 0.13 to 1.00. The transient hyperpolarization at light onset was increased in the response to full-field stimuli (filled arrow) but the transient depolarization at light off was increased in the response to the slit and decreased in response to full-field stimuli (open arrows). (C) After background illumination the response to full-field stimuli relative was greater than to slit stimuli. The slit/full-field response ratio was 0.93. The hyperpolarizing transient at light onset was increased in response to full-field stimuli after background illumination. Stimulus time length, intensities and ordinate details are identical to Fig. 4.



with L-NOLA as in control retinas suggesting that superfusion with L-ARG after L-NOLA caused only a partial recovery. Furthermore, the recovery of the effect after L-ARG superfusion was evident only during the application of background illumination, while the persistent effects of background illumination on receptive-field size showed no recovery from L-NOLA treatment (Fig. 4C). Recovery after L-ARG treatment was evident in 3/3 cells studied. L-ARG itself did not alter the horizontal cell dark resting membrane potential.

Similar results were observed in 2/3 cells when retinas were superfused with another NOS blocker, 100 μ M N^o-nitro-L-arginine methyl ester (L-NAME) (Fig. 6, 7). Background illumination, which normally reduces horizontal cell receptive-field size at first during, and later after, the time the background illumination is on, was ineffective in the presence of L-NAME. After superfusion with L-ARG, background illumination once again reduced horizontal cell responses to full-field stimuli, but this effect was not as marked as in retinas that had not been superfused with L-NAME (Fig. 8, 9). Furthermore, while the effect of L-NAME on the reduction of horizontal cell responses during background illumination did recover after subsequent superfusion with L-ARG, the effect of L-NAME on the persistent effect of background illumination after subsequent superfusion with L-ARG did not recover. No



Figure 6 Effect of background illumination on a horizontal cell from a light-sensitized retina treated with 100 μ M N^G-nitro-L-arginine methyl ester. Prior to background illumination the intensity of the slit (S) was adjusted to be comparable to the response to full-field (FF) stimuli. During and after background illumination the response to full-field stimuli was greater than the response to the slit. Slit intensity $-2.2 \log I_0$, full-field intensity $-3.7 \log I_0$, background intensity $-2.5 \log I_0$. Stimulus time length was 350 msec and ordinate indicates membrane potential in mV.

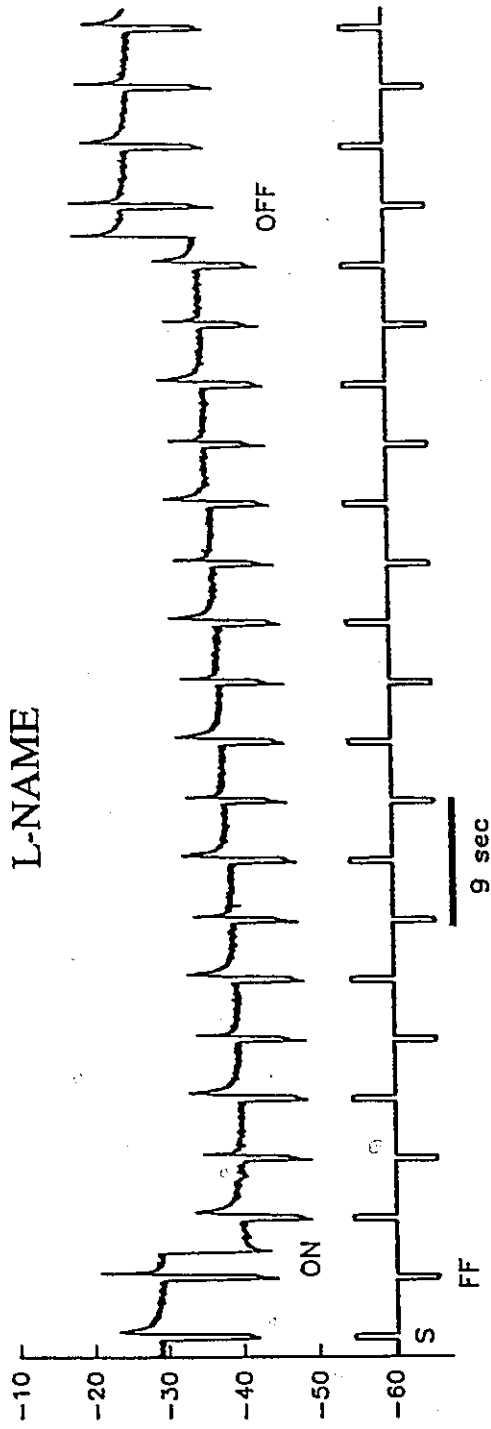


Figure 7 Details of the response waveform of the horizontal cell depicted in Fig. 6. (A) Hyperpolarizing transients at light onset were noted even prior to background illumination (filled arrow) as are depolarizing transients at light off (open arrow). The ratio of slit/full-field response here was 0.83. (B) In the presence of background illumination the horizontal cell was hyperpolarized, the response to stimuli decreased but the response to full-field stimuli was greater than the response to slit stimuli. In fact, the slit/full-field response ratio increased only by 0.07 to 0.90. The hyperpolarizing transient at light onset in response to full-field stimuli was increased in the presence of background light. (C) After the background light was extinguished the response to full-field stimuli was greater than the response to slit stimuli with a slit/full-field response ratio of 0.89. No light-sensitization is apparent because the response to full-field stimuli was less after background light than before. The hyperpolarizing transient at light onset was enhanced in the response to full-field stimuli after background illumination. Stimulus time length, intensities and ordinate details are identical to Fig. 6.

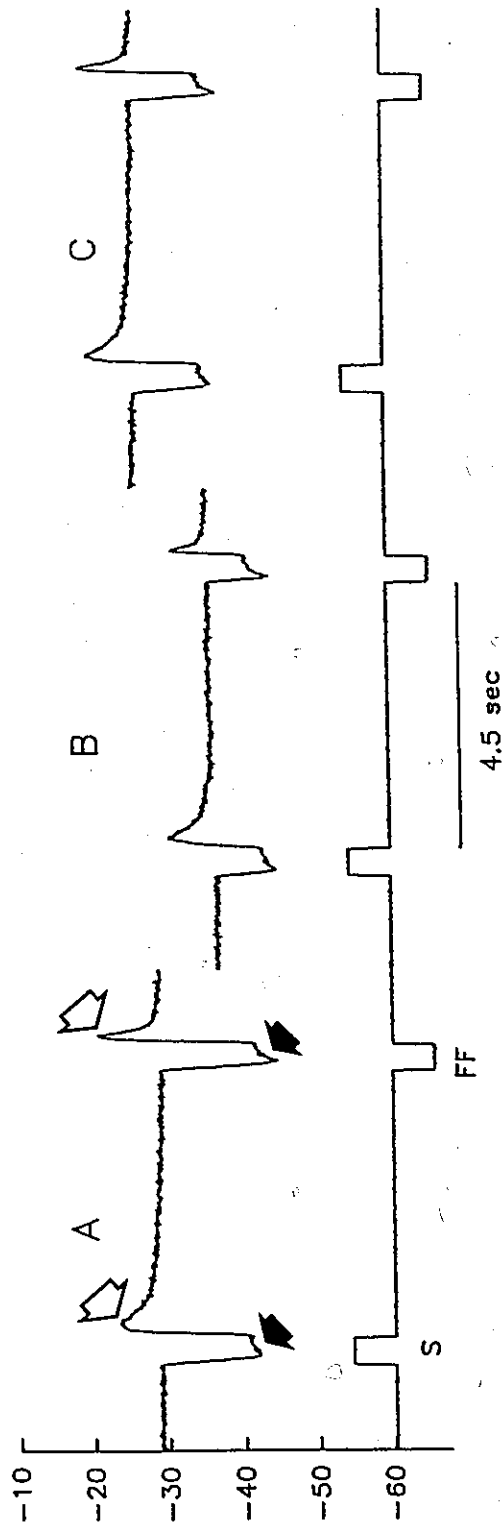


Figure 8 L-arginine partially reverses the effect of N^o-nitro-L-arginine methyl ester on horizontal cell light-adaptation. This was a cell from the same retina as in Fig. 6 but in a horizontal cell impaled after treatment with 100 μ M L-arginine for 20 min. Prior to background illumination the response of to full-field stimuli (FF) was somewhat greater than the response to slit (S) stimuli. During application of background light the response to full-field stimuli became less relative to the response to slit stimuli. After the background light was turned off the response to full-field stimuli was greater than the response to slit stimuli. Slit intensity $-2.4 \log I_0$, full-field intensity $-3.7 \log I_0$, background intensity $-2.5 \log I_0$. Stimulus time length was 350 msec and ordinate indicates membrane potential in mV.

L-ARG RECOVERY FROM L-NAME

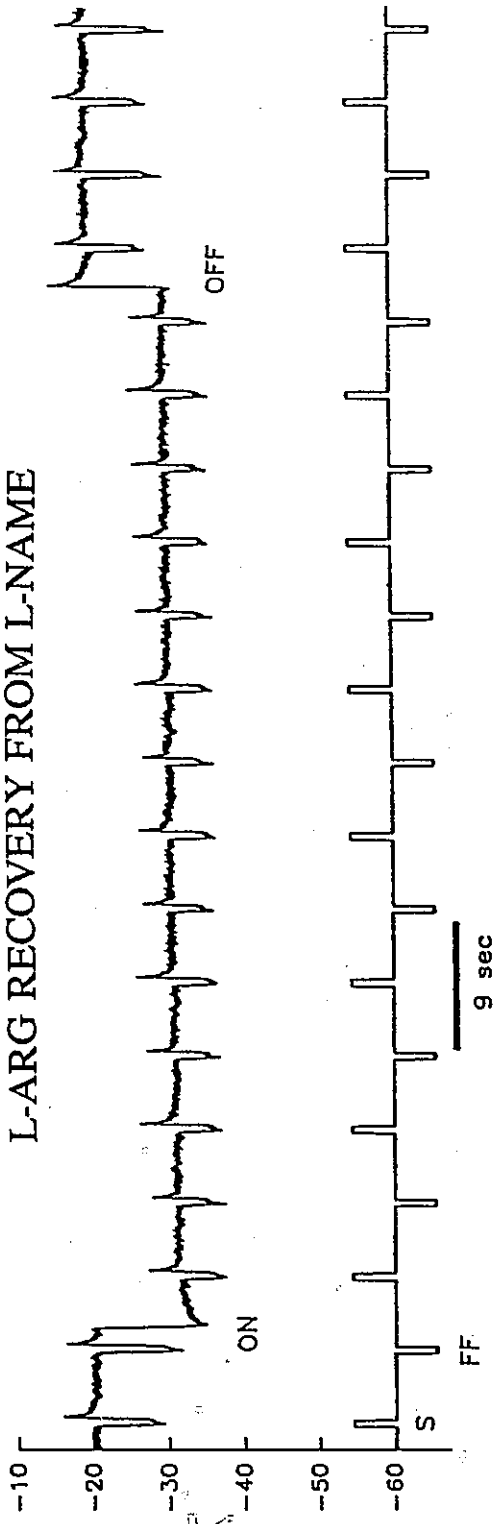
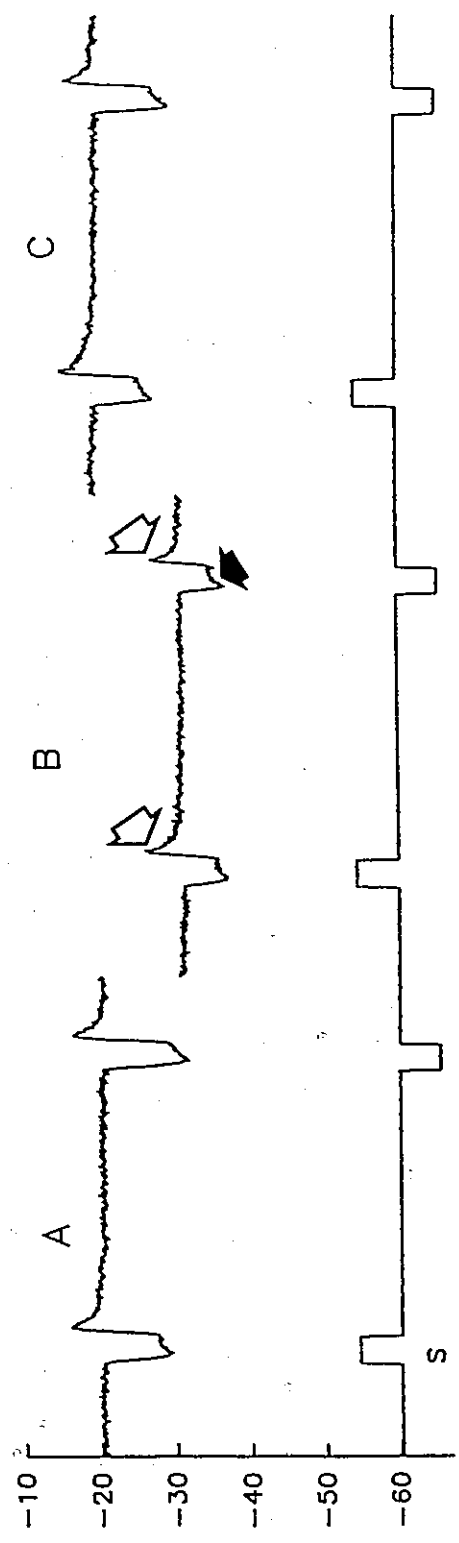


Figure 9 Details of the response waveform of the horizontal cell depicted in Fig. 8. (A) Prior to background illumination the response to full-field stimuli was greater than the response to slit stimuli. The slit/full-field response ratio was 0.81. (B) During presentation of background light the response to full-field stimuli was decreased relative to the response to the slit. The slit/full-field response ratio increased by 0.19 to 1.00. The transient hyperpolarization at light onset was increased in the response to full-field stimuli (filled arrow) but the transient depolarization at light off was relatively unaffected (open arrows). (C) After background illumination response to full-field stimuli relative was greater than the response to slit stimuli. The slit/full-field response ratio was 0.79. The hyperpolarizing transient at light onset was enhanced in response to full-field stimuli after background illumination. Stimulus time length, intensities and ordinate details are identical to Fig. 8.



change of horizontal cell membrane potential was noted during treatment with either L-NAME or L-ARG.

5.3 Observations on changes horizontal cell response waveform after treatment with NOS inhibitors and background illumination

The shape of the waveform of horizontal cell responses to light stimuli changes depending on the light history of the retina. Horizontal cell responses from dark-adapted retinas are typically slow and do not show hyperpolarizing transients at light onset (see Fig. 1, 3, Chapter III). As the retina becomes light sensitized, the response kinetics increase (see Fig. 3, Chapter III). The response kinetics of the horizontal cells recorded in the present study suggest that they were all light-sensitized (Fig. 3, 7). In control retinas, background illumination increased a hyperpolarizing transient at light onset in response to full-field stimuli (Chapter III). NOS inhibitors had no effect on this increase (Figs. 3, 7; Table 1). The proportion of the hyperpolarizing transient contributing to the horizontal cell response to full-field stimulus increased from 0.07 to 0.22 during background illumination. Although this change was statistically significant ($p=.041$), it was reduced compared to the change seen in control retinas (0.08 to 0.41, Table 1, Chapter III). No consistent change in the depolarizing transient response of horizontal cells at light off were noted (Table 2) although the amplitude of the depolarizing transient relative to the

amplitude of the total horizontal cell response did increase in the responses to slit stimuli (Table 3).

5.4 Statistics

Ratios of slit/full-field response amplitude were determined for horizontal cells before, during and after the application of background illumination (Fig. 10). In control retinas the ratio of slit/full-field stimuli response typically rises by 0.35 from a value of 0.83 ± 0.03 prior to light to about 1.18 ± 0.06 during background illumination and increased by 0.8 to 0.95 after background illumination was turned off (Fig. 10, Control). In the presence of NOS inhibitors, the increase in slit/full-field response ratio during and after application of background illumination was decreased to <0.10 (on average 0.02, increasing from 0.85 ± 0.01 to 0.87 ± 0.04) (Fig. 10, L-NOLA or L-NAME). After treatment with L-ARG, the slit/full-field response ratio increased by >0.10 (on average 0.15, increasing from 0.85 ± 0.02 to 1.00 ± 0.02) (Fig. 10, L-ARG). This change was statistically significant ($p < 0.001$) as determined by analysis of variance. There was no significant change in slit/full-field response ratio after the background light was extinguished after treatment with NOS inhibitors (0.82 ± 0.03) or after treatment with NOS inhibitors and subsequent superfusion with L-ARG (0.83 ± 0.01).

Table 1 -- Size of hyperpolarizing transient as a proportion of total horizontal cell response (see Fig. 12, Chapter III) after treatment with NOS inhibitor.

	before background illumination	during background illumination	after background illumination
full-field stimulus	0.07 ± 0.03 n=9	0.22 ± 0.06 n=9 (p=0.041)	0.15 ± 0.03 n=9
slit stimulus	0.07 ± 0.03 n=9	0.07 ± 0.03 n=0	0.08 ± 0.03 n=9

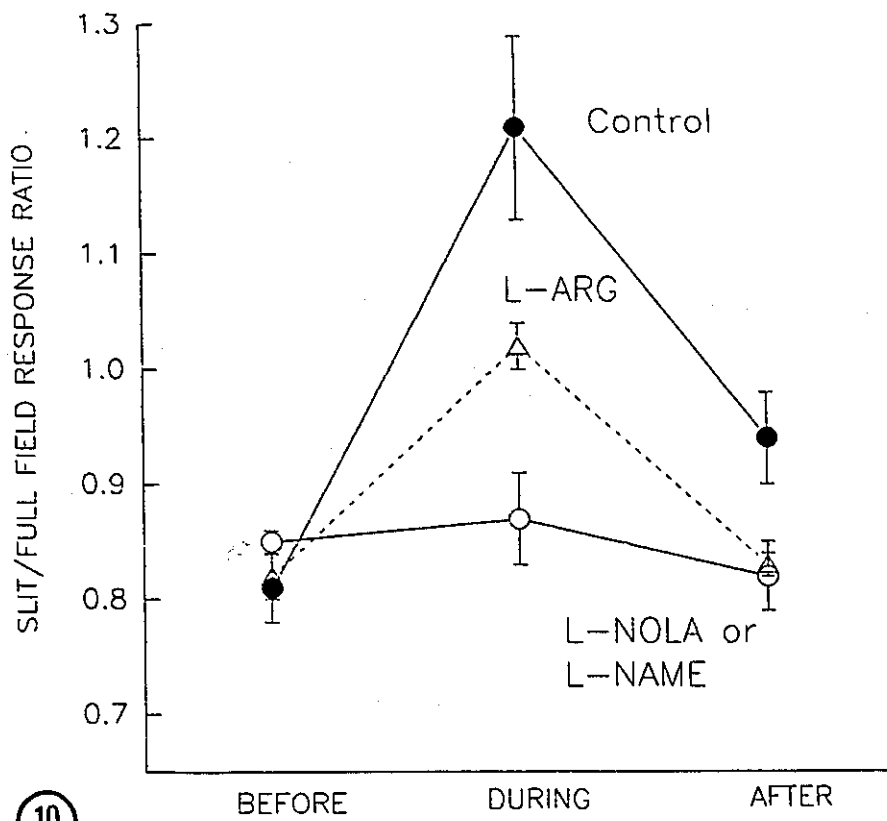
Table 2 -- Change of depolarizing transient (see Fig. 12, Chapter III) after treatment with NOS inhibitor.

	$C_{\text{during}}/C_{\text{before}}$ background illumination	$C_{\text{after}}/C_{\text{before}}$ background illumination
full-field stimulus	0.60 ± 0.08 n=8	0.94 ± 0.02 n=8
slit stimulus	0.99 ± 0.16 n=8	0.98 ± 0.04 n=8

Table 3 -- Amplitude of depolarizing transients relative to maximum horizontal cell response amplitude (see Fig. 12) after treatment with NOS inhibitor.

	before background illumination	during background illumination	after background illumination
full-field stimulus	0.49 ± 0.07 n=9	0.67 ± 0.09 n=9 p=0.120	0.51 ± 0.03 n=9
slit stimulus	0.61 ± 0.06 n=9	1.07 ± 0.15 n=9 p=0.011	0.58 ± 0.09 n=9

Figure 10 Summary of changes in slit response/full-field response ratio before, during and after application of background illumination in the presence of NOS inhibitors and L-arginine. The graph illustrates mean slit/full-field response ratios. In control retinas (filled circles, from Chapter III) the effect of background light increased the slit/full-field response ratio by an average of 0.35 during application of background light and can lead to a persistent change even after the background is turned off. In the presence of NOS inhibitors, there was no significantly increase in the ratio (open circles) during or after background illumination. When retinas treated with NOS inhibitors were subsequently treated with L-arginine, there was a partial recovery of the effect of background light (open triangles). Error bars are standard error of the mean.



10

6.0 Discussion

Previous studies have suggested that NO may play a role in modulating horizontal cell receptive-field size. Intracellular injection of sodium nitroprusside reduces horizontal cell junctional conductance (DeVries and Schwartz 1989; Miyachi, Murakami and Nakaki 1990) and intracellular injection of L-ARG increases horizontal cell input resistance, an effect blocked by inhibitors of NOS (Miyachi, Murakami and Nakaki 1990). In addition, intracellular injection of cyclic GMP, the likely second messenger of NO-mediated effects, into horizontal cells decreases their electrical coupling and increases input resistance (DeVries and Schwartz 1989; Miyachi and Murakami 1991). The results of this study suggest that an NO-mediated mechanism is associated with the light-induced reduction of horizontal cell receptive-field size in the goldfish retina. The effect of background illumination during application of background illumination was reduced by the NOS inhibitors L-NOLA and L-NAME, an effect that was partially reversed by subsequent superfusion with L-ARG. A persistent reduction in horizontal cell receptive-field size, one that continues even after the background light is extinguished and associated with light-adaptation, was also not observed in retinas treated with NOS inhibitor but this effect was not recovered after subsequent superfusion with L-ARG. It is not known why superfusion with L-ARG after L-NOLA or L-NAME did not completely restore the ability of background illumination

to reduce horizontal cell receptive-field size during background illumination or the reduction that usually persists after the background illumination is turned off. Partial recovery with L-ARG during background illumination suggests that superfusion of retinas with 100 μM L-ARG for 20-30 min is not sufficient to reverse the effects of exposures to 100 μM L-NOLA or L-NAME for 10 min. It is also not known if longer exposure to background illumination after NOS inhibitor and L-ARG recovery would have resulted in the persistent changes in receptive-field size observed after background illumination in control retinas.

Physiological studies of the effect of background light on horizontal cells suggest that their receptive-field size is reduced, both during and after background illumination, by a decrease in horizontal cell membrane resistance (Umino, Lee and Dowling 1991; Chapter III). This conclusion comes from the finding that no absolute increase in the response to small spot or slit stimuli is observed, only a relative increase due to the greater decrease in the response to full-field stimuli. However, it is not possible to rule out the possibility that a reduction in gap junction resistance contributes to the change in horizontal cell receptive-field size that was observed. We have previously shown that the persistent change of horizontal cell receptive-field size is accompanied by a decrease of Lucifer yellow dye coupling (Baldrige and Ball 1991). This suggests that horizontal cell gap junction

resistance increases after background illumination. It is not possible to confirm a change in gap junction resistance from our physiological data because the response to both slit and full-field stimuli decreased during and after background illumination. The decrease in responsiveness that was observed could mask an increase in response to slit stimuli due to reduced gap junction coupling.

The NOS inhibitors, L-NOLA and L-NAME, reduced the effect of background illumination on horizontal cell responses to full-field stimuli. This suggests that NO may be involved in the light-dependent reduction of horizontal cell membrane resistance. However, the idea that NO might be involved with changes of horizontal cell membrane resistance is inconsistent with the conclusions of Miyachi, Murakami and Nakaki (1990) who suggested that the effect of injected L-ARG were due exclusively to increased in gap junction resistance because L-ARG had no effect on horizontal cell dark resting membrane potential. Although background illumination reduces the responses of horizontal cell to full-field stimuli, indicating a decrease in horizontal cell membrane resistance, background illumination does not result in sustained changes in horizontal cell dark resting membrane potential (see Chapter III) after background illumination is turned off. Changes in horizontal cell dark resting membrane potential would be expected with changes in synaptic membrane resistance. However, reduced non-synaptic membrane resistance, present at

potentials negative to the dark resting membrane potential (Trifonov, Byzov and Chailahian 1974), could reduce horizontal cell receptive-field size without affecting synaptic membrane resistance at the dark resting membrane potential. Therefore, it is possible that NO affects non-synaptic membrane resistance, and only when the horizontal cell membrane potential is hyperpolarized such as during background illumination.

The NOS inhibitors did not block the reduced responsiveness of horizontal cells to both slit and full-field stimuli after background illumination suggesting that this change is not related to NO. Experiments are being conducted, using dye injection, to determine if NOS inhibitors reduce the effect of background illumination on horizontal cell gap junction coupling.

It has previously been shown that background illumination alters the waveform of horizontal cell responses (see Chapter III). The hyperpolarizing transient usually detected at light onset is enhanced by background light. Such a change has been suggested to be related to increased horizontal cell to cone feedback (Weiler and Wagner 1984). The NOS inhibitors did not block the enhancement of the hyperpolarizing transient at light onset suggesting that this effect of light on horizontal cells is not be affected by NO. Therefore, if the hyperpolarizing transient at light onset is an indication of feedback it is unlikely that feedback is the cause of the

reduced response to full-field stimuli during and after background illumination since NOS inhibitors reduced the effect of background illumination on full-field responses but not the change in the hyperpolarizing transient.

The intracellular target of NO is soluble guanylate cyclase (Katsuki et al. 1977), the activation of which results in an increase in cyclic GMP (Miki, Kawabe and Kuriyama 1977; Yoshikawa and Kuriyama 1980; Förstermann et al. 1990; Reiser 1990; Kiedrowski, Costa and Wroblewski 1992). The cascade of the possible effects of cyclic GMP, mediated by soluble guanylate cyclase, are slow taking from seconds to minutes to have an effect. For example, the classical cyclic AMP-mediated effect of the β -adrenergic receptor on calcium channel conduction in the heart is at least 10^4 times slower than typical fast chemical synaptic transmission, taking tens of seconds to minutes (Nargeot et al. 1983; Fischmeister and Hartzell 1986; Hille 1992). Similarly, the time needed for NO mediated production of cyclic GMP and the subsequent effects of cyclic GMP suggest that the effects of NO should take on the order of seconds to minutes. The reduction of horizontal cell receptive-field size during background illumination follows rapidly with the onset of background illumination. In control retinas, we have found that the reduction in the response to full-field stimuli can occur within < 2 sec (see Fig. 8, Chapter III) after the onset of light. The slow kinetics of the cyclic GMP second messenger system suggests

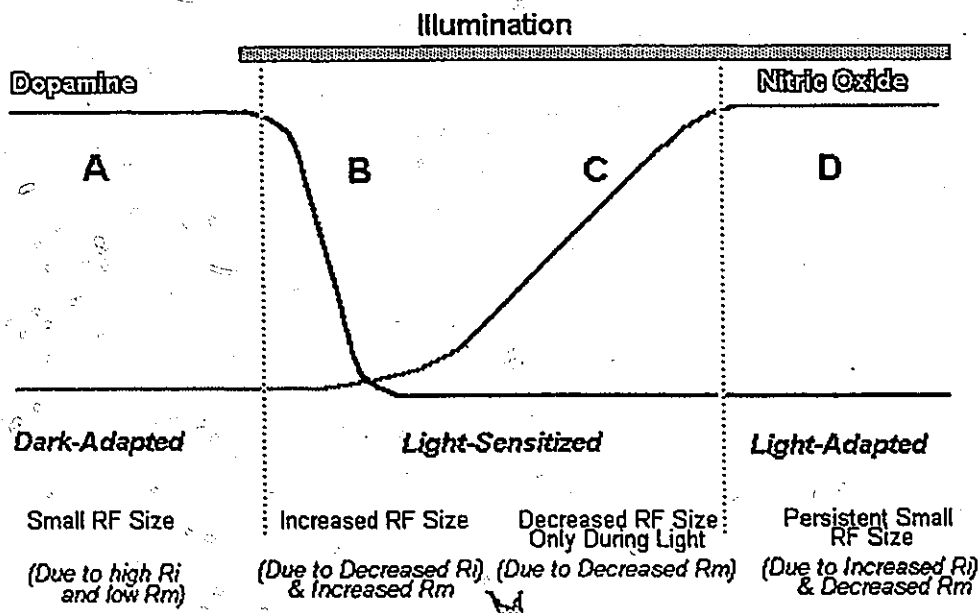
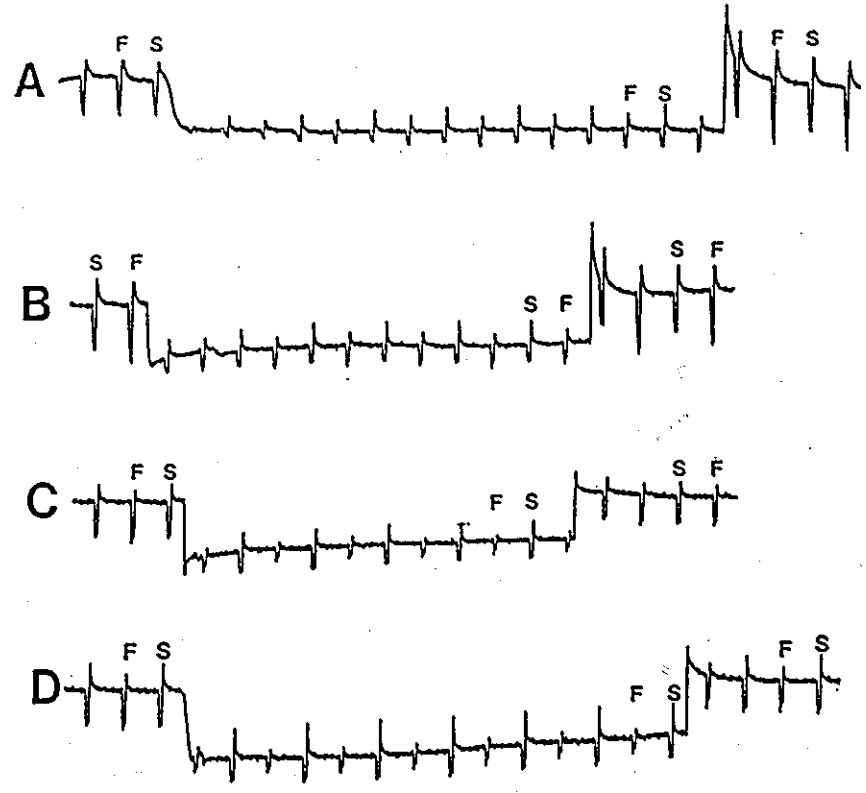
that NO likely acts as a modulator of another mechanism that can rapidly affect horizontal cell receptive-field size rather than being the direct mediator of the effect of background illumination itself. Because there is a rapid change in membrane potential accompanying the onset of background illumination, NO might modulate a voltage-dependent mechanism that affects horizontal cell receptive-field size, such as the voltage-dependent reduction in non-synaptic membrane resistance (Trifonov, Byzov and Chailahian 1974). In such a model NO would be necessary but not sufficient for the light-dependent reduction of horizontal cell receptive-field size observed during background illumination. The receptive-field size of horizontal cells is neither reduced nor increased by background illumination in retinas that have just been shifted from a dark-adapted to a light-sensitized state (Fig. 11B). It is possible that, after a single, brief exposure to background illumination, insufficient NO was produced to prime the horizontal cell mechanism that is sensitive to the change in membrane potential. Exposure to additional background illumination causes the receptive-field size to be reduced suggesting that sufficient NO levels have been produced to activate the voltage-sensitive mechanism that governs receptive-field size during background illumination (Fig. 11C). The time course for the production of NO might also provide an explanation for the delayed persistent change in horizontal cell receptive-field size that occurs after the

retina becomes light-adapted. If NOS activity is increased after exposure to background illumination, it is possible that prolonged or repeated exposure to background illumination would result in sustained levels of NO that are responsible for the persistent effects of light on horizontal cell receptive-field size that are observed (Fig. 11D). Because the horizontal cell is not hyperpolarized after the background illumination is turned off, a persistent change in receptive-field size could not depend on membrane hyperpolarization. It may be, therefore, that the possible role of NO in the persistent change of horizontal cell receptive-field size after background illumination is different from the possible role of NO on horizontal cell receptive-field size during background illumination. The theoretical changes of NO during and after background illumination and the resulting effect on horizontal cell receptive-field size are summarized in Fig. 11.

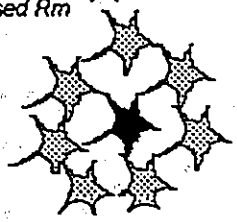
The reduction of turtle horizontal cell coupling by intracellular injection of L-ARG (Miyachi, Murakami and Nakaki 1990) suggest that NOS is located within horizontal cells. This suggestion is supported by the finding that fish horizontal cells are NADPH diaphorase-positive and NOS-immunoreactive (Fig. 1). That horizontal cells are NADPH-diaphorase has also been demonstrated by Liepe, Stone and Copenhagen 1993. Neuronal NOS is a calcium/calmodulin dependent enzyme (Bredt and Snyder 1990) and goldfish

Figure 11 Possible role of NO in the effect of background illumination on horizontal cell receptive-field size. In the dark-adapted retina (A) increased dopamine release increases horizontal cell R_1 and decreases horizontal cell R_m (middle panel) leading to reduced receptive-field size and responsiveness (beginning of (A), upper panel) and reduced dye coupling (lower panel). Presentation of background illumination to dark-adapted retina leads to a (B) light-sensitized retina with increased horizontal cell responsiveness and receptive-field size (end of (A), beginning of (B), upper panel) and dye-coupling (lower panel) due to decreased dopamine release that decreases R_1 and increases R_m (middle panel). Application of background illumination to a retina in early light-sensitization (upper panel, (B)) does not have any effect on horizontal cells because both dopamine and NO levels are low (middle panel). With increasing background illumination (C), NO production increases and activates a voltage-dependent decrease in R_m (middle panel) that reduces horizontal cell receptive-field size during background illumination ((C), upper panel). Additional background illumination leads to a light-adapted retina after increased NO production reduces horizontal cell receptive-field size by a change in R_m or R_1 ((D), middle panel) that reduces horizontal cell receptive-field size ((D), upper panel) and dye-coupling (lower panel) even after background illumination is turned off.

11



Lucifer Yellow Dye Coupling



horizontal cells have also been shown to be immunoreactive for calmodulin (Pochet et al. 1991) but the source of calcium is more problematic. In the rat cerebellum, NOS activity is thought to be increased by the influx of calcium at NMDA receptors or by changes of voltage-dependent calcium currents resulting from non-NMDA glutamate receptors (Garthwaite, Charles and Chess-Williams 1988; Garthwaite, Southam and Anderton 1989; Wood et al. 1990). Although the photoreceptor input to fish horizontal cells is glutamatergic (Murakami, Ohtsu and Ohtsuka 1972; Cervetto and MacNichol 1972; Marc and Lam 1981; Marc et al. 1990), fish horizontal cells do not possess NMDA receptors (Lasater and Dowling 1982; Rowe and Ruddock 1982; Ariel et al. 1984; Ishida et al. 1984). Even more important, however, is that photoreceptor transmitter release is decreased in the light (Trifonov 1968; Trifonov and Byzov 1965; Byzov and Trifonov 1968) resulting in decreased horizontal cell cation conductance (Watanabe 1978; Tachibana 1985). Therefore, even if calcium could pass into horizontal cells through the non-NMDA glutamate receptor-gated non-specific cation channels (Tachibana 1985), such an influx would increase in the dark, not the light. Studies of horizontal cell calcium channels reveal channel inactivation at potentials more negative than -20 mV (Tachibana 1985). Therefore, there is no evidence that calcium conductance of horizontal cells increase with light.

An alternative source of calcium could come from intracellular stores, such as endoplasmic reticulum (Nahorski 1988). Interestingly, it has been reported that NOS is located predominantly at the endoplasmic reticulum (Wolf, Würdig and Schünzel 1992) suggesting that intracellular calcium release may play an important role in the regulation of NOS. Although fish horizontal cells contain endoplasmic reticulum (Yamada and Ishikawa 1965) there is as yet no evidence to indicate that they are a significant site of storage or release of calcium in these cells. In the rat hippocampus, glutamate can induce intracellular calcium release via an inositol 1,4,5-trisphosphate (IP₃) pathway (Murphy and Miller 1988). Although glutamate has been shown to induce phosphoinositide turnover in fish horizontal cells (Weiler, Wenzel and Janssen-Bienhold 1993) it has not been demonstrated that an IP₃ pathway regulates intracellular calcium release in horizontal cells. Furthermore, as in the case of calcium influx, a glutamate-induced release of intracellular calcium would be greatest in the dark, when glutamate release from photoreceptors is increased.

The suggestion that horizontal cells both produce and are affected by NO differs from other models of NO function in the central nervous system. NO has been implicated as a neurotransmitter involved in both long-term potentiation in the hippocampus (Bohme et al. 1991) and long-term depression in the cerebellum (Shibuki and Okada 1991). In both cases, NO

is considered to act as a retrograde neurotransmitter in that NO is released by one cell type and has effects on target cells that are presynaptic to it (O'Dell et al. 1991; Shibuki and Okada 1991). It has been shown that NMDA-induced release of NO from rat cerebellar cells does not elevate cyclic GMP in the NOS-containing cells (Garthwaite, Charles and Chess-Williams 1988). The unusual effect of NO on horizontal cells, resulting from horizontal cell NOS activity, is suggestive of an intracellular second messenger role for NO rather than a transmitter role. An alternative possibility is that another NADPH-diaphorase/NOS-immunoreactive retinal cell is the source of NO affecting horizontal cells such as photoreceptors, ON bipolar cells or amacrine cells (Baldrige, Jamieson and Ball 1993).

In the rabbit retina, NADPH-diaphorase staining is found only in amacrine cells suggesting that amacrine cells are the only source of NO in this retina (Sandell 1985). Recently, Mills and Massey (1993) showed that treatments that should elevate retinal NO reduce the coupling of rabbit A-type horizontal cells as demonstrated by reduced spread of intracellularly injected Neurobiotin. Massey, Mills and de Vente (1993) showed that treatments that elevate NO increase cyclic GMP levels in A-type horizontal cells. Koistinaho et al. (1993) have reported that light increases fos protein, an indicator of synaptic activation, in some rabbit retinal amacrine cells that are also NADPH-diaphorase positive. Taken

together, these results suggest that rabbit retinal horizontal cell coupling can be regulated by NO released from amacrine cells and that the production of NO is increased in the light. However, another recent report (Haberecht et al. 1993) identified NOS-immunoreactivity in an unidentified class of rabbit horizontal cells raising the possibility that NO might be produced by horizontal cells in the rabbit retina.

Because NOS inhibitors and L-ARG were dissolved in the superfusate in our experiments, all retinal neurons were exposed to these treatments. This approach was used because of its simplicity and because a similar superfusion technique has proven effective in the rat hippocampal slice preparation (Böhme et al. 1991). It is, therefore, not known what effects drugs affecting NO release might have on other retinal neurons that might consequently affect horizontal cells. First, it has been suggested that rod photoreceptors contain an NO-sensitive guanylate cyclase that can alter their dark voltage and light response (Venturini et al. 1991; Margulis, Sharma and Sitaramayya 1992; Schmidt, Nöll and Yamamoto 1992; Tsuyama, Nöll and Schmidt 1993). In the present study, treatment with the NOS inhibitors and L-ARG did not alter horizontal cell membrane potential or responsiveness. Because the horizontal cells studied receive input from cone photoreceptors only (Stell 1967), our data do not indicate that the treatments with NOS inhibitors or L-ARG markedly affect goldfish cone photoreceptor cyclic GMP levels. It is

not known if these treatments had any effect on rods. Secondly, Shiells and Falk (1992) have reported that NO can increase cyclic GMP levels in ON bipolar cells of dogfish retina. While it is possible that NOS inhibitors and L-ARG also affect ON bipolar cells, it is not clear how such an effect might influence horizontal cell receptive-field size. Finally, it has been demonstrated that NMDA-evoked release of [³H]-dopamine from striatal slices can be blocked by NOS inhibitors and that NO itself can promote striatal [³H]-dopamine release (Hanbauer et al. 1992). NMDA receptor activation has recently been shown to be important in the regulation of dopamine release from dopaminergic interplexiform cells of the fish retina (Harsanyi, Wang and Mangel 1993). Since horizontal cell receptive-field size has been shown to be affected by dopamine (Teranishi, Negishi and Kato 1984; Mangel and Dowling 1987), this finding might implicate NO in the dopamine-dependent uncoupling of goldfish horizontal cells. While such an involvement may be associated with the reduction of horizontal cell receptive-field size reported in dark-adapted retinas, it is unlikely to play a role in the light-mediated reduction of horizontal cell receptive-field size because it has been shown that the effect of background illumination on fish horizontal cells is dopamine-independent (Baldrige and Ball 1991; Umino, Lee and Dowling 1991).

In summary, the results presented suggest that NO is involved in the light-dependent reduction of horizontal cell receptive-field size in light-sensitized fish retina. It is proposed that the effect of NO is to act as a light-dependent modulator of an unidentified mechanism, possibly voltage-dependent, that rapidly reduces horizontal cell receptive-field size during illumination. Nitric oxide may also be involved in the persistent change of horizontal cell receptive-field after background illumination, but whether the mechanism leading to a persistent change in horizontal cell receptive-field size after background illumination is similar to the mechanism that reduces horizontal cell receptive-field size during background illumination is not clear.

VII. Summary

A great deal has been learned about horizontal cell synaptic connections (Stell 1967) and their responses to light (Svaetichin and MacNichol 1958) since this retinal cell was first identified in 1892 (Ramón y Cajal 1892). Over the last three decades the importance of the large receptive-field size of horizontal cells (Naka and Rushton 1967) in the generation of the receptive-field surrounds of bipolar and ganglion cells (Werblin 1970) has been determined.

Most recently it has been discovered that the receptive-field size of cone horizontal cells is not static but changes depending upon the level of ambient illumination (Mangel and Dowling 1985). It is believed that such changes in cone horizontal cell function result in the change of the size and strength of the receptive-field surround of ganglion cells during dark-adaptation (Barlow, FitzHugh and Kuffler 1957; Raynauld, Laviolette and Wagner 1979). A modulator of horizontal cell receptive-field size, dopamine, has been identified and is suggested to be the mediator of the effects of darkness (Dowling 1986).

A major finding of this thesis is that although the receptive-field size of horizontal cells is reduced by background illumination, it is regulated by a mechanism that does not depend on dopamine (Chapter II). These studies

showed that the effect of light was due, at least in part, to an increase in horizontal cell gap junction resistance because dye-coupling, like receptive-field size, was also decreased by light.

The fact that the effect of background light on horizontal cell receptive-field size is not dopamine dependent (Chapter II) supports the suggestion that dopamine is not released in the light in sufficient concentration to affect D1 dopamine receptors on horizontal cells. This conclusion is consistent with other studies of horizontal cell responsiveness and receptive-field size (see Chapter I, Section 3.10) but not with studies of other aspects of retinal function associated with adaptation that are believed to be dopamine-dependent and suggest that dopamine is released in the light (see Chapter I, Section 3.11).

Doubts have arisen about whether dopamine is really involved in many of the changes in the retina induced by light. Some of these concerns are discussed in Chapter I, Section 3.26. In addition, and as a consequence of this thesis work, (discussed in Chapter II), it was concluded that light-dependent changes of horizontal cell gap junction particle density may not be related to dopamine release *in vivo* and may, therefore, not necessarily be a good indicator of dopamine release in the light.

Two other aspects of horizontal cell function suggested to be regulated by dopamine release in the light, spinule

formation and chromatic sensitivity (recently reviewed by Wagner and Djamgoz 1993), were not studied in the present work. However, one important question this thesis raises is whether the adaptive state of the retina in these studies was truly dark-adapted, as stated, or actually light-sensitized. Recordings from the horizontal cells in these retinas (Kirsch, Wagner and Djamgoz 1991) demonstrated prominent light responses (> 10 mV) suggesting that these retinas were light-sensitized, not dark-adapted. While their data shows that light and dopamine induce horizontal cell spinules and horizontal cell depolarizing responses to chromatic stimuli, it is still necessary to demonstrate that dopamine is the light-dependent signal regulating these events.

The most direct evidence supporting dopamine release in the light are those that directly measure dopamine release (see Chapter I, Section 3.23). However, these studies are not unequivocal in their demonstration of a light stimulated release of dopamine (see Chapter I, Section 3.26). The results of this thesis do not support the evidence which suggest that dopamine is released in the light.

The effect of background illumination on horizontal cell receptive-field size was investigated in the thesis (Chapter III). These studies revealed that retinal horizontal cell activity cannot be classified as only dark- or light-adapted. Physiological studies of horizontal cells suggested there are

at least three adaptational states with different horizontal cell response and receptive-field properties (Table 1.

When background illumination reduces horizontal cell receptive-field size this is due to the effect of light on a light-sensitized retina. This result clarifies a potential source of controversy arising from the finding that light can either increase (Yang, Tornqvist and Dowling 1988a; Tornqvist, Yang and Dowling 1988) or decrease (Shigematsu and Yamada 1988) horizontal cell receptive-field size. This thesis showed that the change depends on which of the three adaptation states the background light is applied to (Table 1).

This thesis has also shown that the effect of background illumination on light-sensitized retina has two components. First, there is a decrease in the receptive-field size during the application of background illumination which, providing there is sufficient exposure to light, is followed by a second change that includes a persistent decrease in receptive-field size after the background illumination is turned off. It is suggested that these two phases of the effect of light on light-sensitized retinas are related because the change during illumination always preceded the persistent change and the persistent change was never observed without a change during illumination.

Table 1 -- Summary of the three states of retinal adaptation.

State	Horizontal Cell Receptive-Field Size	Effect of Background Illumination on Horizontal Cell Receptive-Field Size
<i>dark-adapted</i>	reduced	increases
<i>light-sensitized</i>	increased	decreases
<i>light-adapted</i>	decreased	decreases

The physiological results reported in this thesis suggest that the change in horizontal cell receptive-field size during light-adaptation is due to a decrease in horizontal cell membrane resistance. This conclusion comes from the observation that the horizontal cell response to full-field stimuli decreases during and after background illumination. An increase in horizontal cell gap junction resistance after background illumination, predicted from the decreased Lucifer yellow dye-coupling, could not be demonstrated physiologically since there was no absolute increase in the response to slit stimuli accompanying the decrease in response to full-field stimuli. However, this may be due to the reduction of horizontal cell responsiveness that was observed during and after background illumination that might mask any increase in the response to slit stimuli. This thesis has also found that background illumination alters the waveform of the horizontal cell response to light. These changes are consistent with increased horizontal cell to cone feedback that might also contribute to a change in horizontal cell receptive-field size.

The role of horizontal cells in contributing to the receptive-field surround of bipolar and ganglion cells has been frequently referred to throughout the thesis. As originally suggested by Mangel and Dowling (1985), the loss of ganglion cell surround after dark-adaptation (Barlow, FitzHugh and Kuffler 1957; Raynauld, Laviolette and Wagner 1979) might

be due to reduced horizontal cell receptive-field size and responsiveness. Therefore, according to this model, retinas with light-adapted horizontal cells should have decreased ganglion cell receptive-field surrounds because horizontal cell receptive-field size is decreased. There have not been any studies of bipolar or ganglion cell receptive-fields that make a distinction between the light-sensitized and the light-adapted retina. Therefore, it is not known if the receptive-field surround of bipolar or ganglion cells change after light-adaptation. Furthermore, it is not clear what significance reducing bipolar or ganglion cell surround would have in the light-adapted retina.

Reduced horizontal cell coupling may not necessarily reduce bipolar or ganglion cell receptive-field surround. When horizontal cells are uncoupled the input resistance of the horizontal cell network increases. As a result, even small changes in current, resulting from small changes in cone neurotransmitter release in dim light, would produce a greater potential change than if the horizontal cells were coupled and R_i lowered. Although the reduction of horizontal cell responsiveness after dark-adaptation could eliminate the cone horizontal cell input to bipolar cells, it is not clear why horizontal cell coupling would decrease.

Alternatively, if increased horizontal cell coupling acts to reduce horizontal cell input to bipolar cells, in the light-sensitized retina bipolar and ganglion cell surrounds

should be decreased. After light-adaptation, when horizontal cells receptive-field size is decreased, the receptive-field surround would appear. This is consistent with the idea that under dim light conditions the retinal network is configured for sensitivity at the expense of acuity.

The remainder of the thesis dealt with the identification of a potential mechanism of horizontal cell light-adaptation. Prompted by the immunohistochemical identification of two new putative adrenergic neurons in the fish retina, the possibility that an adrenergic neurotransmitter might provide input to horizontal cells and might regulate receptive-field size during light-adaptation was investigated (Chapters IV and V). In particular, the identification of a new type of interplexiform cell, immunoreactive for phenylethanolamine N-methyltransferase (PNMT), the synthesizing enzyme for epinephrine, raised the possibility of an adrenergic innervation of horizontal cells.

The effect of both norepinephrine and epinephrine on horizontal cells in the fish retina suggested that these adrenergic compounds could affect horizontal cell receptive-field size by acting as agonists at dopamine receptors. Pharmacological studies did not reveal adrenergic receptor-mediated regulation of horizontal cell receptive-field size. Because the effect of background illumination was found to be independent of the action of dopamine and dopamine receptors (Chapter II), the hypothesis that an adrenergic mechanism is

responsible for light-adaptation was rejected. Nonetheless, these studies raised the interesting question of what role the adrenergic neurons may have in the fish retina. For example, if the neurotransmitter of the PNMT-immunoreactive interplexiform cell is epinephrine, and horizontal cells are a target of epinephrine, then it is possible that horizontal cells could be affected in a non-traditional manner in that epinephrine would act at dopamine receptors. It is also not known what conditions may release epinephrine. Furthermore, if epinephrine is not the neurotransmitter of these interplexiform cells, then it is not clear why this cell would be immunoreactive for PNMT or what the actual transmitter might be.

This thesis suggests that the free radical gas nitric oxide may be involved in the process of light-adaptation (Chapter VI). Two inhibitors of nitric oxide synthase reduced the effect of background illumination on horizontal cells in light-sensitized retinas. Partial recovery of the effect of background illumination was observed when the inhibitor-treated retinas were subsequently treated with L-arginine. A persistent change in horizontal cell receptive-field size was not observed after treatment with NOS inhibitor, even after subsequent treatment with L-arginine. However, this may be a consequence of insufficient light exposure or the continued effect of the inhibitor. There is some evidence for the

latter in that the effect during illumination was only partially recovered by L-arginine.

The kinetics of the second messenger system associated with nitric oxide, cyclic GMP produced by soluble guanylate cyclase, is probably too slow to account for the rapid changes seen in horizontal cell receptive-field size while background illumination is on. It was therefore proposed that NO might act as a neuromodulator of another, as yet unidentified, mechanism that can respond quickly to the change in illumination. This model suggests that background illumination induces increased NO production and that, after sufficient light-adaptation, NO affects a rapidly-responding light- or voltage-sensitive mechanism. It was also speculated that as levels of NO rise, after additional background illumination, NO produces the persistent reduction of horizontal cell receptive-field size. This might explain the gradual development of the persistent reduction of horizontal cell receptive-field size after exposure to background illumination.

All of the horizontal cells studied in this thesis, and the resulting discussion of horizontal cell function in the vertebrate retina, refer to effects on cone type horizontal cells. Furthermore, the loss of ganglion cell surround in the fish retina after dark-adaptation was due specifically to the loss of cone-driven surround responses (Raynauld, Laviolette and Wagner 1979). It is therefore important to realize that

these results apply to mechanisms that are active in the mesopic to photopic range of ambient illumination. In the scotopic range, light levels fall below the threshold for cones and the retina becomes rod driven. Under scotopic conditions it is the rod horizontal cells of the fish retina (H4) that respond to light. Although there is some suggestion that dopamine reduces rod horizontal receptive-field size (Yamada et al. 1992), the functional significance of this effect on rod horizontal cells is not clear. Under scotopic conditions rods have been reported to drive both the receptive-field centre and surround of ganglion cells (Adams and Afandor 1971; Beauchamp and Daw 1972). It is possible that rod horizontal cells contribute to the surround of ganglion cells in the scotopic retina. Whether or not there are changes in the strength of the rod-driven ganglion cell surround with changes of ambient illumination within the scotopic range, and if such changes are due to changes in rod horizontal cell activity, remains to be shown.

1.0 Comparison with other vertebrate retinas

Background illumination has been shown to decrease the receptive-field size of horizontal cells in the mudpuppy (Dong and McReynolds 1991, 1992) and turtle (Weiler and Akopian 1992) retina. However, in both cases, the effect of light was blocked by a dopamine receptor antagonist suggesting that in these animals dopamine is released in the light. This conclusion is supported by measurements of endogenous dopamine

release in the *Xenopus* retina (Boatright, Hoel and Iuvone 1989). In the mudpuppy retina, the effect of background illumination also clearly involves an increase in horizontal cell gap junction resistance (R_1), also consistent with the action of dopamine on these retinas (Dong and McReynolds 1991, 1992).

The effect of light on fish horizontal cells, as shown in this thesis (Chapter II; Baldrige and Ball 1991) was not dopamine dependent. Similar results in the fish have been obtained by Umino, Lee and Dowling (1991). There are no obvious explanations why there should be a difference between these different lower vertebrate retinas. One difference is that fish retina possess dopaminergic interplexiform cells whereas amphibian and reptilian retinas are believed to contain only dopaminergic amacrine cells (Witkovsky et al. 1984; Nguyen-Legros et al. 1985b; Kolb et al. 1987; but see Schütte and Witkovsky 1991). However, it is not clear how such a difference in dopamine cell type might explain the difference between adaptation mechanisms in fish and other lower vertebrate retinas.

Dong and McReynolds (1991) have also shown that the effect of light on mudpuppy horizontal cell receptive-field size could be blocked by the glutamate receptor antagonist 2-amino-4-phosphonobutyrate (APB). Because APB blocks the photoreceptor to ON bipolar cell synapse (Slaughter and Miller 1981), Dong and McReynolds suggested that the dopaminergic

amacrine cell of the mudpuppy receives excitatory input from ON bipolar cells. This is consistent with the light-stimulated release of dopamine since ON bipolar cells are depolarized in the light.

In the study and discussion of the effect of nitric oxide, it was argued that second messenger systems are inherently slow, at least compared to conventional fast chemical synaptic transmission. The effect of dopamine on horizontal cells is mediated by the second messenger cyclic AMP and, therefore, should also act slowly (Dowling 1986). In the amphibian and reptile retina, where dopamine is thought to be released from amacrine cells in the inner retina (Piccolino and Demontis 1988), the kinetics of dopamine action would be slowed by the time needed for dopamine diffusion from the inner to outer retina. Therefore, if the effect of background illumination on horizontal cell receptive-field size in these retinas is as rapid as in the fish, it is difficult to explain how dopamine could act so quickly.

There is no information regarding the effect of background illumination on horizontal cell receptive-field size in mammalian retina. However, tyrosine hydroxylase activity and dopamine synthesis are reported to be increased in the rat and rabbit retina during steady light (Iuvone et al. 1978; Parkinson and Rando 1983) as is endogenous dopamine release in the rabbit (Godley and Wurtman 1988). This leads to the speculation that horizontal cell receptive-field size

in these animals is reduced in the light by dopamine. However, such an argument could have been applied to the fish retina where dopamine release is reported to increase during steady light (Dearry and Burnside 1989; Weiler, Kolbinger and Kohler 1989; Kolbinger et al. 1990) but the reduction of horizontal cell receptive-field size in the light does not appear related to dopamine (Chapter II; Baldrige and Ball 1991; Umino, Lee and Dowling 1991).

In every vertebrate retina examined to date horizontal cell receptive-field size is decreased in the light. However, whether the mechanism of this change is dopamine-dependent or not continues to be a subject of controversy. The identification of light-sensitization, an intermediate step between dark-adaptation and light-adaptation, in the fish retina was an important finding in the interpretation of the effects of light on fish horizontal cells (Chapter III). Whether such an intermediate step exists in other vertebrate retinas should be investigated before the effects of background illumination on horizontal cells from these species can be fully evaluated.

Nitric oxide has been implicated as a mediator of horizontal cell receptive-field size in the turtle retina (Miyachi, Murakami and Nakaki 1990). There is also evidence from the rabbit retina that nitric oxide may regulate horizontal cell coupling and, therefore, receptive-field size (Mills and Massey 1993). However, these studies have not

determined under what lighting condition nitric oxide is produced in the retina. Nonetheless, there is some indirect evidence that suggest that nitric oxide production is elevated in the rabbit retina during light (Koistiniallo et al. 1993).

2.0 Significance to human vision

2.1 Fish retina as a model of human retina

Much less is known about the physiology of the human retina than is known about the retina of fish. Studies of retinas from other primates has contributed to the understanding of the human retina but studies of retinas from other mammals have indicated there are important differences between primate retina and the retinas of cats and rabbits. Although there are certainly important differences between the fish retina and the human retina, there are many features that the fish retina have in common with human retina that are not shared by all mammalian retinas.

One of the key similarities between fish and human retina is the fact that both retinas are Duplex and capable of colour vision. Like the goldfish retina, the primate retina contains rods and three types of cones (Nunn, Schnapf and Baylor 1984; Baylor, Nunn and Schnapf 1987; Schnapf et al. 1988). Three morphological types (HI, HII, HIII) of horizontal cells in the human retina have been described as well as the photoreceptor input to each type (Kolb, 1991). The HI horizontal cell has an axon but the other two types do not. In primates, there is no distinct rod horizontal cell (unlike fish) with rod input

going to the axon terminal of the HI horizontal cell. However, it has been suggested from work in the cat retina that the rod and cone inputs to the HI horizontal cell are separated by an electrically isolated axon (Nelson 1977). In fact, in recordings from primate HI horizontal cells (Dacheux and Raviola 1990) the responses from the somata and the axon terminal appear to be driven exclusively by cone and rod input, respectively. Therefore, as in fish, at the level of the horizontal cells the rod and cone pathway are distinct. The study of Dacheux and Raviola (1990) also demonstrated that the primate HI horizontal cell is a luminosity-type cell responding with graded hyperpolarizations to all wavelengths similar to the fish H1 horizontal cell.

The contact of each horizontal cell with one cone type in the goldfish retina (Stell and Lightfoot 1975) does not appear to be the case in the human retina. In the human retina the horizontal cells receive mixed cone input with the HI cell receiving input from all cone types, the HII cell receiving input from long- and middle-wavelength cones and the HIII cell receiving input from the short- and middle-wavelength cones (Kolb 1991). Although recordings have not yet been made from HII or HIII primate horizontal cells, the pattern of cone (long- & middle- vs. short- & middle-) input does suggest the possibility that the HII and HIII cells of the human, like the H2 and H3 horizontal cells of the fish, are colour-coded.

The dendrites of horizontal cells in the primate retina are located both centrally and laterally within the synaptic terminal of cone photoreceptors (Dowling and Boycott 1966). This general pattern is similar to that in goldfish (Stell and Lightfoot 1975) where it was suggested that the central elements are excitatory and the lateral elements inhibitory. This arrangement explains the response patterns of goldfish horizontal cells to chromatic stimuli. However, until the chromatic response patterns of the HII and HIII horizontal cells are determined it will not be possible to know if a similar situation exists in the primate.

Gap junctions have been identified between horizontal cells in primate retina (Raviola and Gilula 1975). It is presumed, therefore, that the receptive-field size of primate horizontal cells is larger than their dendritic field. However, the extent of the horizontal cell receptive-field has not yet been reported for primate although large receptive-field sizes have been identified in other mammalian retinas (Nelson 1977; Dacheux and Raviola 1982).

In primate retina there are three types of bipolar cells. One type exclusively contacts rods and there are two types of cone bipolar cells. The "midget" bipolar cells contact cones within invaginations of the cone terminal but other bipolar cells make contacts along the flattened base of the cone terminal and are termed "flat" bipolar cells (Dowling and Boycott 1966). From the position of the axon terminals of

these bipolar cells within the inner plexiform layer it has been suggested that the midget bipolar cells are ON cells while the flat bipolar cells are of the OFF type (Famiglietti and Kolb 1976; Nelson, Famiglietti and Kolb 1978).

The arrangement of synaptic contacts within the cone terminal in the human retina is sufficiently similar to that in the goldfish retina to suggest that the electrophysiological properties of bipolar cells of the two should also be similar. That is, the colour-coding and centre-surround receptive-field organization of human bipolar cells may be very similar to that in the fish. However, it has not been possible to record electrophysiologically from primate or human bipolar cells to test this hypothesis.

Evidence from other mammalian retinas, such as rabbit, suggest that horizontal cells contribute to the formation of the receptive-field surround of ganglion cells and, therefore, presumably bipolar cells (Mangel and Miller 1987; Mangel 1991). However, other physiological studies have suggested that amacrine cell input in the inner plexiform layer contributes to the bipolar cell surround in mammals (Dacheux and Raviola 1986; Nelson and Kolb 1983). Although it is true that bipolar cells in human retina receive significant amacrine cell input (Kolb and DeKorver 1991) it has also been suggested, from electroretinographic data, that spectral opponency exists in the outer plexiform layer of the monkey (Sperling 1986). While it is not yet possible to determine

how similar the construction of bipolar and ganglion cell receptive fields are in fish and primate retina, there are some indications of significant similarity.

In summary, the fish and human (primate) retina have much in common. Both retinas are Duplex and in both there is a separation of rod and cone information at the level of the horizontal cells. This is not true of many other mammalian and vertebrate retinas where the retinas can be rod-dominated or rod and cone inputs to horizontal cells are not segregated at all. As previously discussed, it would be interesting to determine if the construction of bipolar and ganglion cell receptive-field surrounds in primate retina are a product primarily of amacrine cell or, like the fish, horizontal cell input.

2.2 Dopamine, horizontal cells and human vision

Another striking similarity between the teleost and human retina is presence of dopaminergic interplexiform cells (Dowling and Ehinger 1978; Frederick et al. 1982). Dopaminergic interplexiform cells have also been identified in New World monkeys retinas (Dowling, Ehinger and Floren 1980; Holmgren 1982; Nguyen-Legros, Moussafi and Simon 1990), possibly in the rat retina (Savy et al. 1989), and only a few cells have been identified in the cat retina (Oyster et al. 1986; Kolb et al. 1990). However, in other mammalian retinas, including those of rabbits and cats, dopamine has been either exclusively or primarily localized to amacrine cells (Törk and

Stone 1979; Holmgren-Taylor 1982; Oyster et al. 1985; Tauchi, Madigan and Masland 1990). Dopaminergic interplexiform cells make specific contacts onto horizontal cells (Dowling, Ehinger and Floren 1980; Yazulla and Zucker 1988) whereas dopamine release from amacrine cells must presumably diffuse from the inner to outer retina to affect horizontal cells (Piccolino and DeMontis 1988). The functional significance of the difference between interplexiform and amacrine cell release of dopamine is not clear. Nonetheless, the fact that human retinal horizontal cells may be innervated directly by dopaminergic interplexiform cells raises the possibility that the action of dopamine in this retina is similar to that described in the fish.

It has been suggested that individuals suffering from Parkinson's disease, where dopaminergic neurons in the midbrain are lost, might also have visual deficits due to the loss of retinal dopamine (for review see Bodis-Wollner 1990). One of the principle visual deficits discovered in Parkinsonian patients is a reduction in contrast sensitivity, but there is not complete agreement that these differences are significant (Harris, Calvert and Phillipson 1992). These results have been interpreted to indicate that the dopamine deficiency decreases surround inhibition of bipolar and ganglion cells (Bodis-Wollner 1990). This suggests that dopamine, which reduces horizontal cell receptive-field size,

acts to increase bipolar and ganglion cell surround in the human retina.

This conclusion is not consistent with the model that relates decreased horizontal cell coupling with the decrease of bipolar and ganglion cell receptive-field surround (Mangel and Dowling 1985; Dowling 1987). However, it is consistent with the model, discussed above (and in Chapter I, Section 2.21, Fig. 7C), that associates decreased horizontal cell coupling with the presence and strength of the ganglion cell receptive-field surround.

A recent study has reported that dopamine content and dopaminergic interplexiform cell number is decreased in retinas of Parkinsonians (Nguyen-Legros et al. 1993). While it is likely, therefore, that dopamine levels are decreased in the retinas of Parkinson's patients, further study is needed before a specific connection with horizontal cell function can be made with certainty. It may turn out that the most important consequences of reduced dopamine in the retina of Parkinsonian patients is the loss of dopaminergic input to cells other than the horizontal cells. There is some indirect evidence to suggest that this may be the case. In the fish retina, the effects of dopamine on horizontal cells are mediated by a D1 dopamine receptor (Dowling 1986). It has been reported that some of the same changes in visual function observed in Parkinson's patients can occur in patients treated with the D2>D1 dopamine receptor antagonist haloperidol

(Bartel et al. 1990; Stanzione et al. 1991) or the selective D2 antagonist sulpiride (Stanzione et al. 1992). Therefore, visual deficits in Parkinsonian patients may be due more to the absence of dopamine input to cells having D2 dopamine receptors rather than putative D1 dopamine receptors on human horizontal cells.

Because studies in fish suggest that dopamine plays a critical role in the adjustment of the retina to different levels of ambient illumination, it would be of interest to test various parameters of human vision in Parkinson's patients under different states of adaptation. The object of these studies would be to determine if differences in the ability of the retina to shift from one adaptational state to another are affected by a loss of dopamine.

2.3 This thesis and human vision

The findings of this thesis suggest that the state of the retina may be more than just simply light- or dark-adapted. On the basis of studies of horizontal cells, this thesis demonstrated that background illumination first increases the receptive-field size of horizontal cells from dark-adapted retina but, with additional light exposure, shift the retina to a light-adapted state where receptive-field size is decreased. Therefore, in studies of the human visual system with respect to adaptation, as in the study of Parkinson's disease discussed above, the adaptation state and light history of the retina may turn out to be critically important.

The thesis also suggested that nitric oxide may play a role in horizontal cell light-adaptation. In the same way that studies of patients treated with dopaminergic drugs have suggested dopamine-dependent alterations of the visual system, it would be compelling to study the vision of patients treated with drugs, such as nitroglycerin or other nitrovasodilators, that might elevate nitric oxide levels in the retina.

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