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SYNAPTIC BASIS OF ROD-CONE INTERACTION IN THE VERTEBRATE RETINA

by

Thor Eysteinsson

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy The City University of New York

1988

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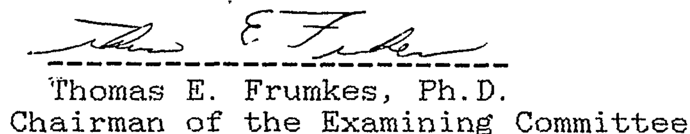

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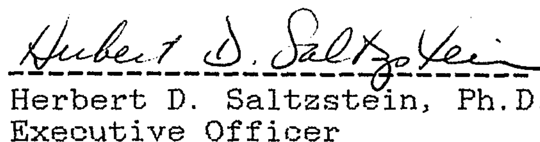

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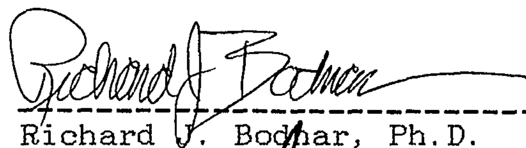

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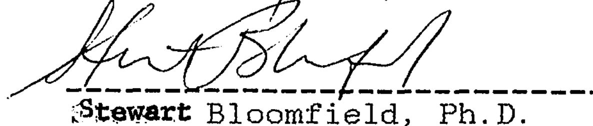

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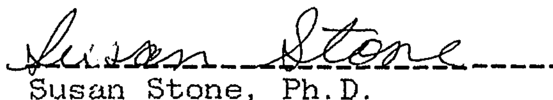

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Abstract

Synaptic Basis of Rod-Cone Interaction in the Vertebrate Retina

by

Thor Eysteinsson

Advisor: Dr. Thomas E. Frumkes.

Frumkes and Eysteinsson (1987) described a type of rod-cone interaction in amphibian retina which they called suppressive rod-cone interaction (SRCI). The intracellular response from most types of retinal neurons was obtained in response to spatially focal stimuli flickering at frequencies >4 hz which amphibian rods cannot follow; therefore, the flicker response reflects cone activity. Dim, spatially diffuse, rod-stimulating backgrounds enhance flicker responsiveness by as much as 800%. Frumkes and Eysteinsson attributed this effect to a tonic inhbitroy influence of the cones upon the horizontal cells (HCs) which is removed by selective light adaptation.

The synaptic basis for SRCI was investigated by means

of intracellular recording from retinal neurons in the superfused eyecup of the mudpuppy, Necturus maculosus. In the distal retina, specific synaptic actions were evaluated by superfusing pharmacological agents with delimited, well characterized effects. Amino acid analogues which selectively block the photic response of HCs (e.g., D-O-Phosphoserine (DOP), Kynurenic Acid (Kya)) block SRCI in cones and bipolar cells. Lead chloride, which selectively blocks the rod input into horizontal cells, blocks SRCI. All these agents enhance the amplitude of the flicker response to levels only seen in the presence of an adapting field. These results are consistent with the model. In contrast, 2-amino-4-phosphonobutyric acid (APB), which blocks the photic response of depolarizing bipolar cells, has no influence upon SRCI in HCs or Off-cells. 1 mM Dopamine reduces electrical coupling between HCs and has little influence upon flicker responsiveness in the dark, but reduces background enhancement of flicker in horizontal and bipolar cells, suggesting that SRCI may depend upon HC electrical coupling.

Some sustained ON- and OFF-ganglion and amacrine cells display SRCI. DOP, and KyA have a similar effect to that observed in bipolar cells, suggesting that SRCI merely reflects an expression of an outer plexiform layer circuit. But SRCI in OFF amacrine and ganglion cell was

in some cases enhanced by APB, indicating that SRCI in the distal retina is further modified by inner plexiform layer circuitry.

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Many thanks to my friends and co-workers in the lab, Dr. Frank Naarendorp, and Ms. Noreen Denny, who were always frendly and helpful.

Finally, I dedicate this dissertation to my parents, Eysteinn Gudmundsson and Valgerdur Gudleifsdottir. They taught me to be persistent when things are difficult, and to try to do my best.

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Chapter 1: General Introduction.

Psychophysical Statement of the Duplicity Theory

The duplicity theory of vision was originally proposed by Schultze in 1866 on the basis of comparative anatomical study of different vertebrate retinae (for reviews, see Hecht, 1937; Barlow, 1972; Gouras, 1972). As it is usually stated, two different types of photoreceptors with distinct functions mediate vision. The cones are responsive to stimuli at higher (photopic) luminance levels while the rods are responsive to stimuli under lower (scotopic) luminance levels.

As stated by modern psychophysicists, the term "duplicity theory" amounts to a collection of empirical facts which relate to known anatomical and physiological differences between the two different photoreceptor types. Scotopic vision involves high absolute sensitivity, low spatial and temporal resolution, lacks color vision, and saturates at relatively low intensity levels. In contrast, photopic vision, involves lower absolute sensitivity, high spatial and temporal resolution, color vision, and does not saturate except perhaps at extremely high levels of illumination (Wysecki and Stiles, 1967; Barlow, 1972; Boynton, 1979).

Although there is an overall difference in the range of illumination over which rods and cones operate, there is an overlapping mesopic range (from approximately 1 to 1000 trolands) over which rods and cone operate simultaneously. Traditionally, it was believed that even under mesopic conditions, rods and cones function independently of one another (e.g., Hecht, 1938; Rushton, 1962). But in recent years, it has become increasingly evident that rod and cone related vision can interact under mesopic levels of illumination. Throughout this thesis, the term rod-cone interaction will be used to indicate an interaction between rod- and cone-related vision somewhere within the visual system, and does not necessarily imply a direct neural connection between these two photoreceptor types.

Since the early 1970s, an overwhelming body of psychophysical evidence indicates a wide variety of conditions under which rod-cone interaction occurs in human vision. Color vision experiments show that under mesopic conditions, rods strongly influence hue and saturation judgements (Stabell & Stabell, 1973; Trezona, 1972). Increment threshold experiments suggest that rod and cone signals often summate. This summation of signals can be discerned when one photoreceptor system is selectively adapted with a steady background, which in turn raises the threshold for the other system (e.g.,

Frumkes and Temme, 1977; Temme & Frumkes, 1977). Rods and cones can also summate together to produce a threshold sensation (Drum, 1982; Frumkes, Sekuler, Barris, Reiss & Chapula, 1973).

Suppressive Rod-Cone Interaction: a psychophysical perspective.

The wide range of psychophysical situations under which it has been possible to demonstrate rod-cone interaction suggests that there may be more than one basic type or mechanism. Using flicker techniques, two different types have been discerned. MacLeod (1972) and van den Berg and Spekreijse (1977) showed that flicker signals generated by rods and cones could either destructively interfere with each other or add together in order to produce a larger overall signal. This type of rod-cone interaction has recently been referred to as "quasiliner" or summatory (Frumkes, Naarendorp, Eysteinsson, Denny, & Goldberg, 1985) as it probably involves the convergence of a like polarity input stemming from the activity of both photoreceptor types. Indeed, such rod-cone interaction has been observed at every level of the neuroretina including the photoreceptors themselves (Fain, 1975; Nelson, 1977). In fact, the original MacLeod (1972) study was essentially

replicated by means of extracellular recording from cat ganglion cells (Rodiek and Rushton, 1976).

Recently, a second type of rod-cone interaction has been described using flicker. Cone mediated flicker sensations are greatly suppressed by selective rod-dark adaptation and enhanced by selective rod light-adaptation (Alexander and Fishman, 1984; Coletta and Adams, 1984; Goldberg, Frumkes, and Nygaard, 1983). This type of rod-cone interaction has recently been referred to as Suppressive Rod-cone Interaction (SRCI) as it is believed to reflect the suppressive influence of dark-adapted rods upon cone pathways (Frumkes and Eysteinsson, 1987). By removing this suppression, selective rod-light adaptation enhances cone-mediated flicker.

In recent years, SRCI has been studied in human observers quite extensively by using either psychophysical or ERG procedures. These experiments have revealed several general features of SRCI. First, action spectra show that SRCI is, quite clearly, an influence of rod adaptation upon the sensitivity of the long wavelength cone mechanism (Goldberg, 1983; Goldberg, Frumkes & Nygaard, 1983; Coletta and Adams, 1984). A number of control experiments using counterphase procedures (Goldberg, 1983;
Goldberg, Frumkes & Nygaard, 1983) clearly show that it cannot involve the activity of a rod-flicker signal and thus, must be distinctly different than the summatory

rod-cone interaction described by MacLeod (1972). SRCI is also relatively small in magnitude with low frequency flicker and increases in magnitude to a >1 log unit effect as flicker frequency increases (Goldberg, Frumkes & Nygaard, 1983; Arden and Hogg, 1984). In addition, SRCI is highly influenced by spatial parameters, and can be demonstrated at virtually every retinal position including the fovea (Coletta and Adams, 1984; Alexander and Fishman, 1984). In general, its magnitude is greatest with small stimuli and it cannot be observed with Ganzfeld stimuli (Goldberg, Frumkes & Nygaard 1983; Arden and Frumkes, 1986, but see Alexander and Fishman, 1986).

Psychophysical research in normal humans is unlikely to pinpoint the neural locus for a mechanism. However, even without considering neurophysiological data from lower vertebrates, human data suggests that SRCI is mediated by the distal retina, which includes the outer nuclear layer and outer plexiform layer. Distal is used in this case in relation to the center of the eye. First, SRCI has been documented by means of electroretinographic (ERG) procedures in humans (Arden and Frumkes, 1986) as well as various lower vertebrates (e.g., Hood, 1972 in frog; Loew and Arden, 1985 in cat and dog). Arden and Frumkes (1986) showed that SRCI must be present by the site of the b-wave component generator of the ERG, i.e. at the level of the bipolar and Mueller cells in the

retina. Second, SRCI is lacking in protanopes, individuals with a genetic defect causing an absence of the red-cone photopigment (Goldberg & Frumkes, 1983; Alexander & Fishman, 1983; Coletta & Adams, 1985). Although it is quite possible that these "color-blind" subjects have neuroretinal as well as photopigment abnormalities, there is no evidence that this could involve structures outside the distal retina. Third, Alexander and Fishman (1985; 1986) report the cases of two individuals with stationary night blindness who show rod-receptor potentials, but no rod b-wave or any behavioral signs of rod vision: yet, these individuals demonstrate SRCI. This suggests that the deficit in these individuals involves the synaptic connection from the rods to rod-bipolar cells but that portion of the rod-neural activity which interferes with cone pathways is unimpaired. Fourth, individuals with a variety of retinal pathologies show distinct abnormalities in SRCI. For example, patients with retinitis pigmentosa that lack rod function do not show SRCI (Alexander & Fishman, 1985), while such patients that have preserved rod function (type II retinitis pigmentosa) show rod-cone interaction (Arden & Hogg, 1985).

To summarize, the data from human subjects suggest that SRCI is retinal in origin. It most probably involves post-receptoral neurons, but neurons that are distal to the bipolar cells. In order to further specify

the underlying neural mechanism, several different laboratories have studied SRCI by means of intracellular investigations in subhuman species. Before considering these, the overall functioning of the vertebrate retina will be reviewed

Overview of Vertebrate Retinal Organization

The overall anatomical organization of the vertebrate retina was first described by Santiago Ramon y Cajal (1893), using the Golgi silver impregnation method and light microscopy. Five basic classes of neurons - photoreceptors, horizontal cells, bipolar cells, amacrine cells and ganglion cells - and glial cells (called Mueller cells) form a relatively orderly structure of rows of cell bodies, called nuclear layers. These nuclear layers are separated by zones of synaptic contacts that are called plexiform layers.

Photoreceptors are the light-sensitive element of the retina, and form the distal-most layer of cell bodies, i.e., the photoreceptor layer. They release an excitatory transmitter maximally in darkness (Trifonov & Byzov, 1965), and hyperpolarize in a graded fashion in response to light (Werblin & Dowling, 1969). The light-evoked hyperpolarization reduces the release of the transmitter (Miller & Schwartz, 1983). The photoreceptors have their perikarya in the outer nuclear

layer, but have also been found to have contacts with other photoreceptors by means of gap junctions or electrical synapses (Raviola & Gilula, 1975) and possibly by means of chemical synapses (Mariani & Lasansky, 1986). However, their most often seen connections in the outer plexiform layer is as the presynaptic element in synapses made with second order neurons.

Bipolar, horizontal, and amacrine cells all have their perikarya in the inner nuclear layer. Their synaptic processes are confined to the plexiform layers. Horizontal cells have processes that extend widely but only within the outer plexiform layer, and photoreceptors synapse with these processes. Horizontal cells are depolarized in the dark and respond to light stimuli with a large graded hyperpolarization (Werblin & Dowling, 1969; Kaneko, 1970), which would suggest that they maximally release their neurotransmitter in the dark (Miller & Dacheux, 1976). Horizontal cells make synaptic contacts onto bipolar cell dendrites, at least in some species (Dowling, Brown & Major, 1966; Dowling, 1979), and with each other via gap junctions (Stell, 1967; Kaneko, 1971). This indicates that horizontal cells are likely to be electrically coupled to each other as since confirmed by an electrophysiological study by Lamb (1976) and a large number of more recent investigations. There is physiological evidence for feedback synapses from horizontal cells to photoreceptors (i.e., since

Baylor, Fuortes & O'Bryan, 1971), which is of particular interest for the present study: such synapses, however, have rarely been observed anatomically (but see Sakai and Naka, 1986).

Bipolar cells are the route for transmission of visual information from the outer plexiform layer to the inner plexiform layer. Their axon terminals are presynaptic to the dendrites of ganglion and amacrine cells. Ignoring chromatic factors, bipolar cells can be functionally classified into two different categories, depending upon the polarity of their photic response to a small spot of light centered over their receptive field center. Some bipolar cells hyperpolarize (hyperpolarizing bipolar cells or HPBCs), while others depolarize (depolarizing bipolar cells or DPBCs) in response to center illumination, due to different opposite conductance changes underlying the response (see Saito, 1987 for review). Both types of bipolar cells exhibit center-surround antagonism (Werblin & Dowling, 1969), such that DPBCs hyperpolarize and HPBCs depolarize in response to an annulus. This surround mechanism is thought in part to reflect horizontal cell input to the bipolar cells, and/or possibly an inhibitory influence of horizontal cells upon photoreceptors (see Miller, 1979 for review). In addition, the receptive-field properties of bipolar cells are probably influenced by feedback of amacrine cells (Miller, 1979). In general, the fundamental

receptive properties of the third order neurons, the amacrine and ganglion cells, are thought to reflect an excitatory input from the two different bipolar types. Thus, ON- amacrine and ganglion cells receive an excitatory input from the DPBCs, OFF-cells receive an excitatory input from the HPBCs, while ON-OFF cells receive an excitatory input from both (Naka, 1976; Miller, 1979).

Amacrine cells have their processes in the inner plexiform layer, and have synaptic contacts with other amacrine cells and ganglion cell processes in that layer, in addition to receiving bipolar input. Ganglion cell axons form the optic nerve and the final output from the retina. One cell type in the vertebrate retina was briefly described in the classic anatomical work of Ramon y Cajal (1893), but has received great attention in recent years. This is a neuron that has its perikaryon in the inner nuclear layer, among the amacrine cell bodies, and has processes extensively in the inner plexiform layer, but has also processes in the outer plexiform layer (Ehinger, Falck & Laties, 1969; Gallego, 1971; Dowling & Ehinger, 1975). Gallego termed this cell type the interplexiform cell (Gallego, 1971), and it was later observed by using the Falck-Hillarp histofluorescence method, that they contain dopamine (Dowling & Ehinger, 1975).

Neuropharmacology of Vertebrate Retina

An increasing amount is being learned about the neuropharmacology of the vertebrate retina. A good deal of this information may be species specific, and I will only review findings relevant to the present study. There is general agreement that photoreceptors release glutamate or a closely related analogue as its neurotransmitter (for review, see Miller and Slaughter, 1986). These physiological and pharmacological studies indicate that neurons postsynaptic to photoreceptors have non-NMDA-type of glutamate receptors, and the distinction between types of receptors classically described in spinal cord (e.g., by Watkins and Evans, 1981) does not apply well in the retina (Miller and Slaughter, 1986). But it is likely that the three types of second order neurons in retina have distinct subtypes of glutamate receptors (Miller and Slaughter, 1986). Wheither or not that is correct, it is clearly established in the retina of the mudpuppy, the species used throughout this thesis, that the response of second order neurons can be selectively blocked by different glutamate analogues. 2-amino-4-phosphonobutyric acid (APB) only blocks the response of the depolarizing bipolar cells (Slaughter and Miller, 1981). Piperidine dicarboxylic acid (PDA) (Slaughter and Miller, 1983) or Kynurenic acid (KyA) (Miller, Slaughter,

Coleman & Massey, 1986) block both the horizontal and hyperpolarizing bipolar cells. Finally, D-O-Phosphoserine (DOP) attenuates the response of the horizontal cells (Slaughter and Miller, 1985). It should be stressed that in the distal retina, DOP, PDA and kynurenic acid act as antagonists to the neurotranmitter released by the photoreceptors: in contrast, APB acts as an agonist. In addition to glutamate receptors, it is likely that NMDA-type of receptors exist on third order retinal neurons (Slaughter & Miller, 1983; Lukasiewicz & McReynolds, 1985).

In recent years the role of dopamine in the retina has received considerable attention. Dopamine has been localized in amacrine and interplexiform cells by the Falck-Hillarp method (Dowling and Ehinger, 1975; Adolph, Dowling and Ehinger, 1980). Physiologically, Negishi and Drujan (1978) found that dopamine reduces the receptive fields of horizontal cells. The effective receptive field of horizontal cells is enlarged by the electrical coupling of adjacent horizontal cells (Kaneko, 1971). It has been found in several species that the fluorescent dye Lucifer yellow diffuses between horizontal cells, while dopamine restricts the diffusion of the dye to one horizontal cell (Teranishi, Negishi & Kato, 1984; Piccolino, Neyton and Gerschenfeld, 1984). It is therefore likely that dopamine uncouples horizontal cells, as first suggested by Negishi and Drujan (1978).

This is supported by data from pairs of isolated horizontal cells, were dopamine was found to reduce the conductance of the gap junctions between the pairs (Lasater & Dowling, 1985). Dopamine therefore seems to regulate the responsiveness of horizontal cells (see Mangel and Dowling, 1987 for further dicussion of dopamine).

Several laboratories have also indicated that several different divalent ions might influence selectively the action of rods or cones. In particular, cobalt (Evans, Hood & Holtzman, 1978), lead, and mercury (Fox and Sillman, 1979; Tessier-Lavigne, Mobbs & Attwell, 1985) each seem to have a much greater influence upon rod- than cone-driven activity. Although the underlying pharmacology and biochemistry are poorly understood, it is clear that the overall influence of these ions involves several distinct effects, including a reduction in the rod receptor potential (Tessier-Lavigne, Mobbs & Attwell, 1985). In the present study, I used lead chloride to selectively block rod activity. The pharmacological information of greatest interest to the present study is summarized in Table 1.

The reader may feel that the forgoing overview is lacking in that it does not mention inhibitory neurotransmitters, particularly GABA. For example, in certain species,

Table 1.

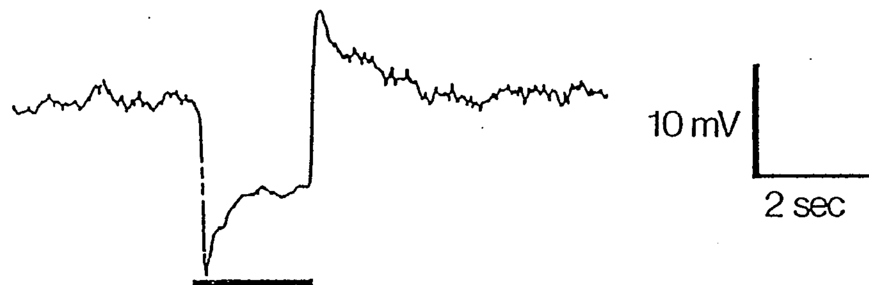
Drug	Action	Reference.
Lead Chloride (Pb)	Selectively Blocks Rods	Fox & Sillman, 1979
Piperidine Dicarboxylic Acid (PDA)	Blocks Hyperpolarizing Bipolar and Horizontal Cells	Slaughter & Miller 1983a
Kynurenic Acid (KyA)	Like PDA above but more potent and cheaper	Miller, Slaughter, Coleman & Massey, 1987
D-O-Phosphoserine (DOP)	Attenuates Horizontal Cell Responses	Slaughter & Miller 1985a
2-Amino-4-Phosphonobutyric Acid (APB)	Blocks Depolarizing Bipolar Cell Response	Slaughter & Miller 1981

there is overwhelming evidence that some classes of horizontal cells (HCs) release GABA (Lam, Lasater & Naka, 1978; Ayoub & Lam, 1985; Yazulla, 1983). GABAergic HCs have been implicated as those likely to feed back onto cones in a variety of species (Lam, Lasater, & Naka, 1978; Stone & Witkovsky, 1984; Lasater & Lam, 1984). However, it is quite clear that in mudpuppy, the species used in the present thesis, most horizontal cells are NOT GABAergic (Miller, Frumkes, Slaughter & Dacheux, 1981). But the transmitter released by most horizontal cells is unknown.

SRCI in the Amphibian Retina

Although SRCI has been explored most extensively with psychophysical procedures in humans, it has also been studied electrophysiologically. Prior to the work from our laboratory, a number of studies reported a very similar phenomenon using ERG techniques (Granit and Riddell, 1934; Dodt and Jessen, 1960; Hood, 1972; Fatechand, 1978). More recently, Frumkes and Eysteinsson (1987) reported SRCI in the clawed toad, Xenopus and the mudpuppy, Necturus. A typical record, from a mudpuppy hyperpolarizing bipolar cell, is shown in figure 1. The top trace shows a response to a spatially diffuse, 2 second duration light flash of 520 nm and log quanta incident cm-2 sec-1 of 9.95. The cell showed a

Figure 1. Intracellular recordings from a mudpuppy hyperpolarizing bipolar cell. The upper trace shows a response to a dim, diffuse 2 second flash. The lower trace shows the response by the same cell to a focal red LED flicker in the presence and absence of a dim, diffuse 520nm rod-adapting stimulus, whose presence is indicated by the downward DC shift of the stimulus monitor. Note the large increase in the flicker amplitude during background, indicating SRCI.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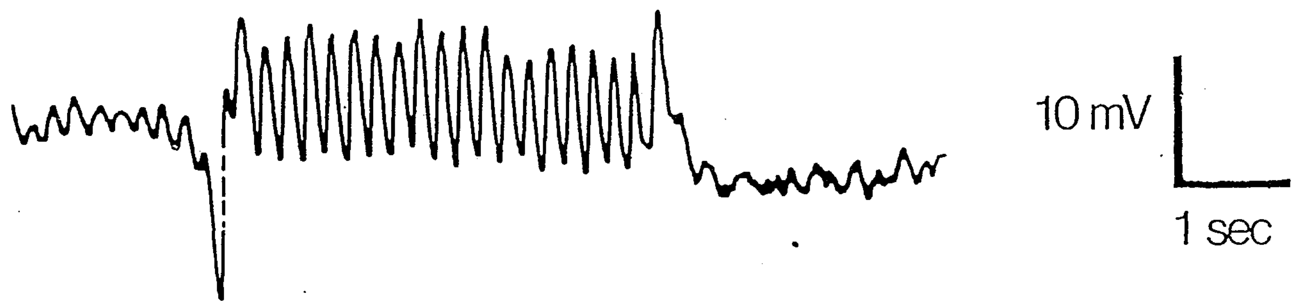
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clear center-surround mechanism when tested. The lower set of traces illustrate an experiment that demonstrates SRCI in this cell. The upper trace is the response, while the lower trace is the output from the stimulus monitor. The stimulus trace shows that a 5Hz flicker stimulus was continuously presented. This stimulus stemmed from a red light emitting diode (LED), was 430um in diameter, and centered with respect to the recording electrode. It had a quantal flux of log quanta incident cm-2 sec-1 of 10.47 and was sinusoidally modulated at 96%. The downward DC shift of the stimulus monitor represents presentation of a diffuse background. In this case, it was 3 seconds in duration, 520nm in wavelength, and had a quantal flux of log quanta incident cm-2 sec-1 of 9.56. Before the presentation of the background, the cell shows a small response to flicker. Presentation of the background causes an overall depolarization, an effect which is explained in the results section, and is attributable to stimulation of the receptive field surround. More importantly, this increases the amplitude of the flicker response, an effect which rapidly reverses when the background is removed. Frumkes and Eysteinsson (1987) showed that this increase in amplitude of cone-driven flicker is due to selective photic stimulation of rods, which is refered to as selective rod light adaptation through this text. Since they also showed that amphibian rods cannot follow flicker frequencies >2

hz, this means that rod light adaptation enhances cone driven responses, and hence, is evidence for SRCI.

In horizontal cells, SRCI has been demonstrated using sinusoidally flickering red light as a test stimulus for cone input (Frumkes & Eysteinsson, 1987). A similar effect has been found in Xenopus horizontal cells by Witkovsky & Stone (1983, 1987) using a red test stimulus that was presented in the presence or absence of a 491 nm rod-adapting background. The background enhanced the cone-driven response to the red (676 nm) stimulus. By recording from a tiger salamander cone while electrically stimulating a neighbouring rod, Attwell, Werblin, Wilson and Wu (1983) found a sign-reversing pathway for rods to influence cones. It is possible that this effect relates to SRCI.

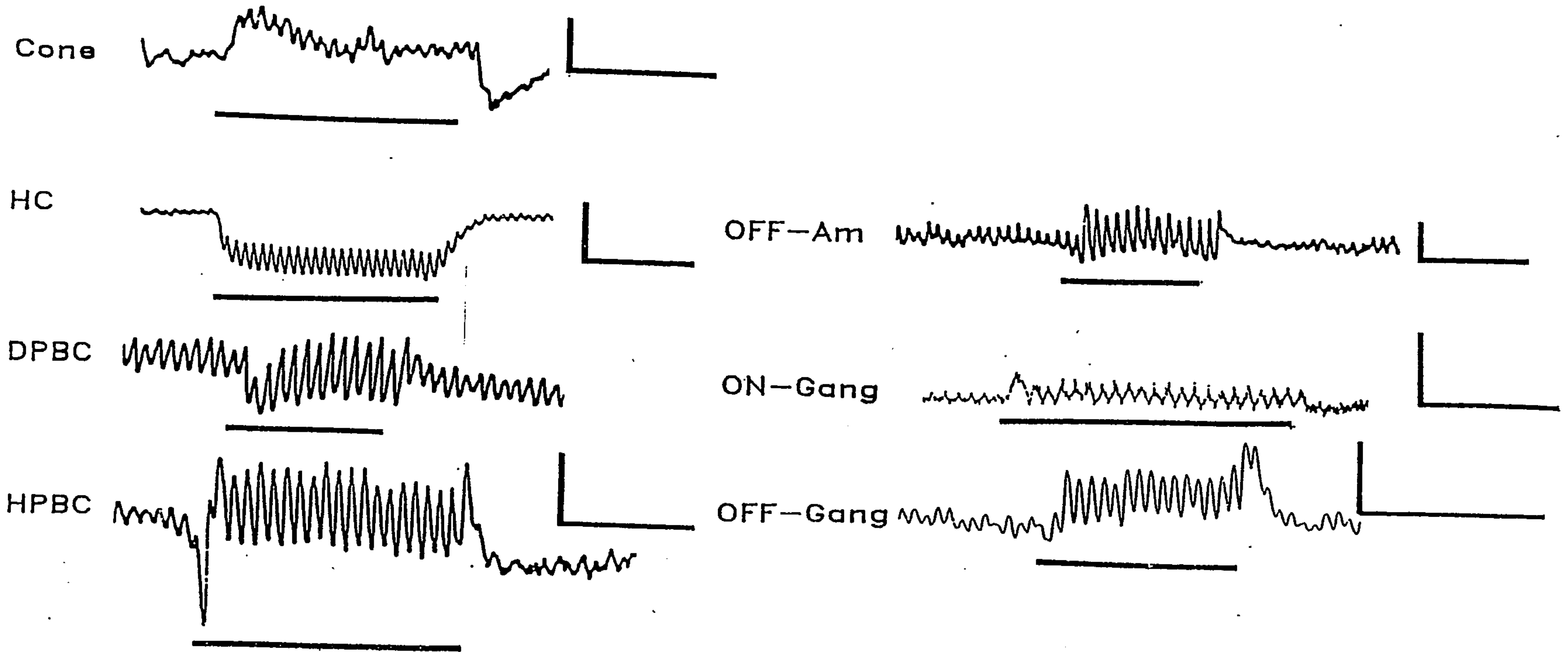
Although other investigators have studied the same or a similar phenomenon in amphibian retina, the paradigm provided by Frumkes and Eysteinsson (1987) has one distinct advantage: the use of rapid flicker provides a signal which is specific to cones. Using this flicker technique, we found in both Xenopus and mudpuppy horizontal cells a rod-adapting background enhanced cone drive responses as much as 800%. In horizontal cells, SRCI is highly dependent on the size and frequency of the flickering stimulus, and the state of adaptation (Frumkes, Eysteinsson & Arden, 1985; Eysteinsson &

Frumkes, 1985; Frumkes & Eysteinsson, 1987). As the size of the flicker stimulus is increased, the relative amount of the enhancement produced by the background decreases. Furthermore, as the frequency of flicker in increased, the relative amplitude of the response enhanced by the background increases (Frumkes & Eysteinsson, 1987). The effect of background clearly involves rod-cone interaction, since it adheres closely to the spectral sensitivity of the green-absorbing rod photopigment, so the enhancement of the response to cone flicker is dependent on the adaptation of rods. In addition, SRCI can only be recorded from scotopically or mesopically adapted neurons, and not photopically adapted units (Frumkes & Eysteinsson, 1987; Pflug & Nelson, 1987).

SRCI can be recorded from retinal neurons other than horizontal cells, as summarized in figure 2. Recordings from distal neurons are shown on the left of the figure, and illustrate that SRCI can be observed in cones, horizontal cells, and depolarizing and hyperpolarizing bipolar cells. This effect can be recorded in many third order neurons, particularly on- and off-spiking amacrine and ganglion cells with sustained response properties. No similar background enhancement effect is observed in color-opponent cells and rods, or in most on-off amacrine and ganglion cells.

Recently, SRCI has also been recorded intracellularly from cat horizontal and bipolar cells

Figure 2. Summary of results from intracellular recordings in various classes of retinal neurons that show SRCI. The left side of the figure shows recordings from a cone and second order neurons, while the right side shows recordings from amacrine and ganglion cells. A small red flickering stimulus was presented in each case, and a diffuse green rod-adapting background superimposed, which caused an increase in the amplitude of the flickering response. The background enhancement is unrelated to the polarity of response to the background. The ON- and OFF-ganglion cells were recorded from the <u>Xenopus</u> retina; all others from mudpuppy.



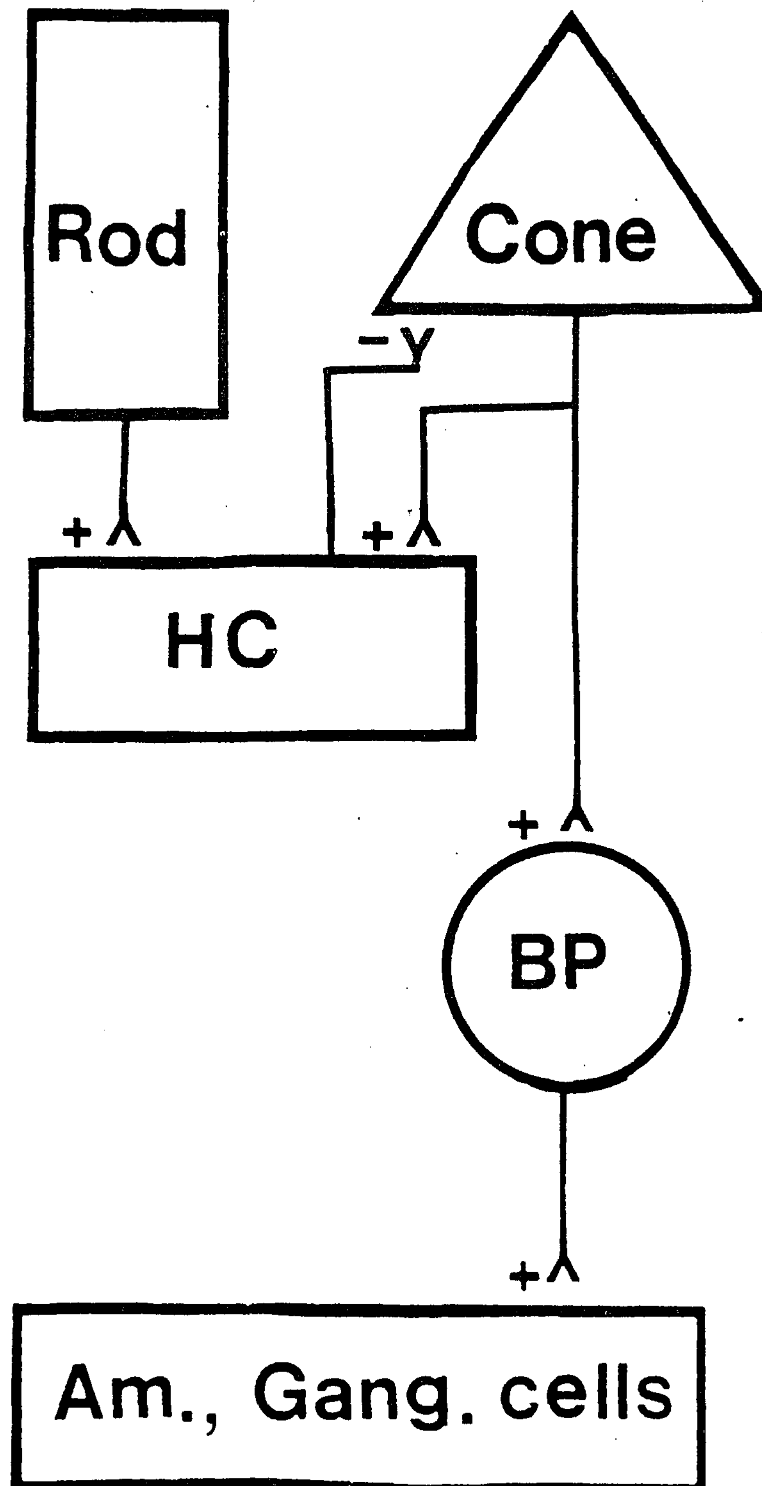
Calibration: 10 mV and 2 sec.

(Pflug & Nelson, 1987), as well as by ERG procedures in humans (Arden and Frumkes, 1986) and cat and dog (Loew and Arden, 1985). Although there is no direct proof, it is therefore possible that SRCI as studied by means of psychophysical procedures in humans and neurophysiological procedures in amphibians may have the same underlying mechanism. Indeed, this possiblity is suggested by the similar spatial and temporal limitations for SCRI obtained by means of psychophysical procedures in humans, and by electrophysiological recordings from cat and amphibian horizontal cells (Eysteinsson, Frumkes, & Denny, 1987; Frumkes, 1987). But for the following parts of this dissertation, SRCI refers only to suppressive rod-con interaction in the amphibian retina.

Tenative Model for SRCI in Amphibian Retina

Since SRCI can be observed in virtually all cone-driven distal retinal neurons including the cones themselves, there are a limited number of possible physiological mechanisms which could mediate this effect. Eysteinsson and Frumkes (1985) proposed the simple model indicated in figure 3. As shown, rods and cones are presynaptic to the horizontal and bipolar cells, but only cones receive inhibitory feedback from horizontal cells.

Figure 3. A proposed model for mechanism underlying SRCI. Rods and cones have excitatory input into horizontal cells while horizontal cells have an inhibitory input only into cones. The rod input into horizontal cells modulates the cone input into horizontal cells, bipolar cells and subsequently third order neurons, collectively represented by the large square at the bottom of the figure.



Rod
Cone
-γ
+
+
HC
+
BP
+
Am., Gang. cells

Given this situation, it would be expected that the ambient level of illumination upon the rods would determine the amount of inhibitory feedback onto cones. Specifically, selective rod-light adaptation causes a hyperpolarization of rods. Assuming that neurotransmitter release is maximal in a depolarized state, it logically follows that this hyperpolarization will lead to a decrease in release of the excitatory rod neurotransmitter, and consequently a hyperpolarization of horizontal cells, and a reduction in the release of inhibitory HC neurotransmitter upon cones. In other words, the adapted state of rods alters the set-point of the otherwise closed feedback loop in the pathway from cones, to horizontal cells to rods.

Although there are a number of problems with this model, which will be addresed in the discussion section, it served as the impetus for the present study.

Rationale for the Present Study.

There are a number of ways the model in figure 3 could be put to an experimental test. In the present study, I primarily used the pharmacological agents summarized in table 1. I used the different types of pharmacological agents to block specific portions of the pathway outlined in fig. 3: if such agents were not

previously studied in mudpuppy, I first indicated their specific action by subsidiary experiments before using them to study SRCI. Thus, I would expect that agents which block the rod input to horizontal cells (lead chloride) or both the rod- and cone-photic response of horizontal cells (D-O-Phosphoserine, Piperidine Dicarboxylic acid, Kynnurenic acid) should block SRCI. Since they all block the effect of photoreceptor neurotransmitter upon horizontal cells, they duplicate the effect of selective rod-light adaptation and should enhance flicker over control levels. Thus, after demonstrating that lead selectively influence rod responses, I used it study rod-cone interaction. If the model in figure 3 is correct, lead should block SRCI: since both lead and selective rod-light adaptation both prevent the release of rod neurotransmitter, lead should also enhance flicker responsiveness over control levels. Second, I used either Kyn, DOP, or PDA to block the light response of horizontal cells. In cones and bipolar cells (and indirectly, in third order neurons), this should also enhance flicker in the dark and block the influence of selective rod light adaptation. I also used several other pharmacological agents which have no counterparts in figure 3, but which for a number of reasons, might also influence SRCI either in distal or proximal retina. I used APB since a complex interplay between on- and off-pathways has recently been proposed to account for many

temporal response properties in mudpuppy retina (Belgum, Dvorak, McReynolds & Miyachi, 1987; Coleman and Frumkes, 1983). I also used dopamine since it is well known to influence spatial properties of horizontal cells in a number of species (Negishi & Drujan, 1978; Piccolino, Neyton, & Gerschenfeld, 1984) and since spatial parameters are well known to limit SRCI (Frumkes & Eysteinsson, 1987; Pflug & Nelson, 1987).

Chapter 2: Methods.

A) Subjects.

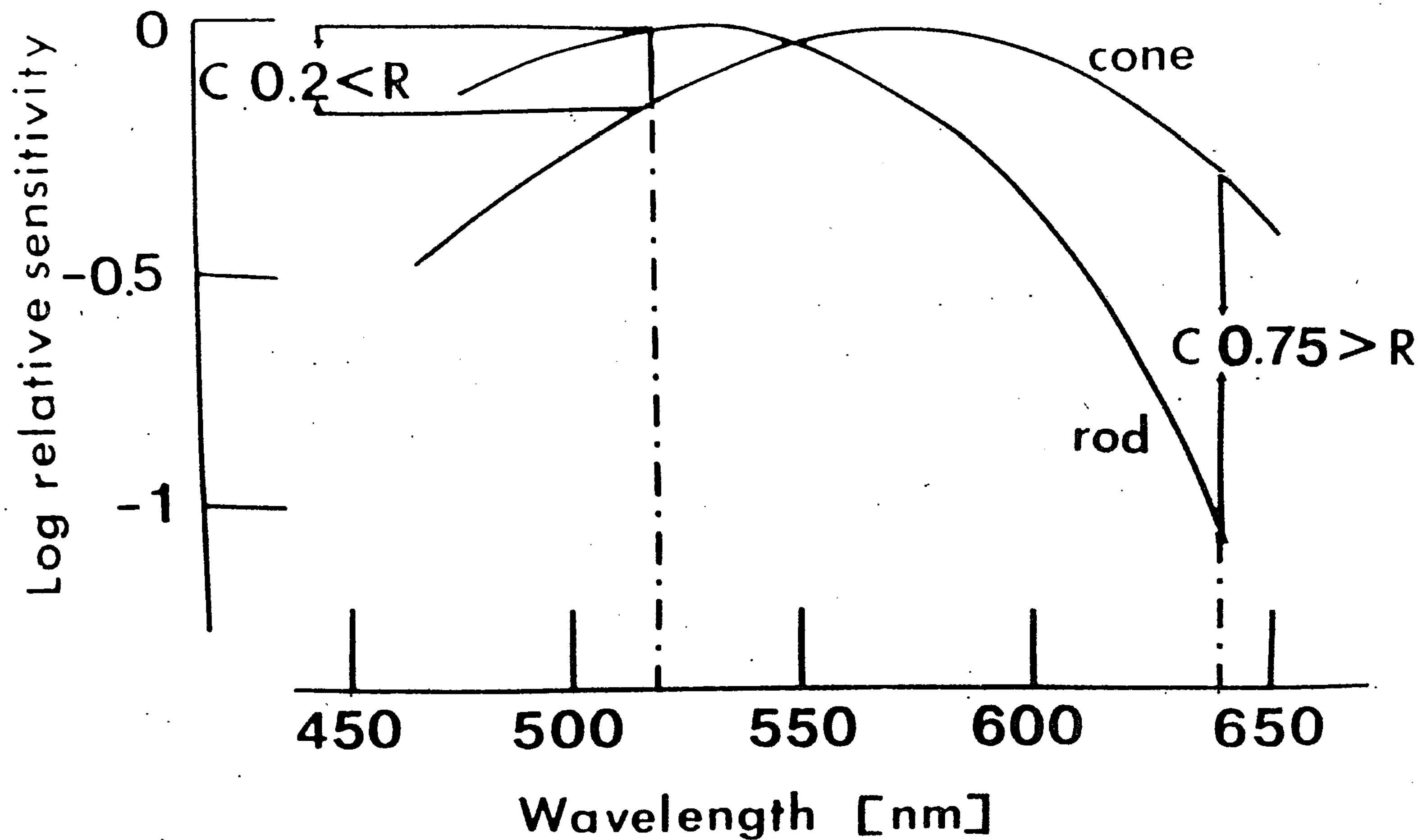
Most of the data in this study are recordings from adult mudpuppies (Necturus maculosus), approximately 15-20 cm in length which were obtained from commercial vendors. This species was used since it is possible to record with relative ease from most retinal neurons which have been characterized both physiologically and pharmacologically in many previous studies. The mudpuppy retina does have some disadvantages. It is optimally responsive only from late fall to early spring, and there seems to be very little rod input into second-order neurons during other seasons (Frumkes & Eysteinsson, 1987) which makes work on rod-cone interaction impossible. Also, the response of the mudpuppy retina to flicker is very sluggish (Karwoski & Proenza, 1978; Frumkes, Coleman & Nicotera, 1981). However, it proved possible to work around these disadvantages. The advantages of mudpuppy as a subject for this study are that most of its retinal cell types are easy to record from, and the pharmacology of the retina has been extensively studied.

The absorption spectra of mudpuppy photoreceptors and the synaptic connections of the photoreceptors have been previously characterized (Dowling and Werblin, 1969; Fain, 1975; Werblin and Dowling, 1969). The mudpuppy retina has three different photoreceptors: a green-absorbing rod which is maximally sensitive at 525nm, a single cone with a maximum sensitivity at 572nm, and a double cone, which has a principal and accessory outer segments. The principal member also has a maximum spectral sensitivity at 572nm (Wald, Brown & Gibbons, 1963; Fain, 1975). Figure 4 shows the absorption spectra for the two known photopigments in mudpuppy retina. All second-order neurons in mudpuppy receive direct input from green-absorbing rods and single cones (Fain, 1975).

B) Dissection.

The animals were quickly decapitated and double pithed by sectioning the spinal cord between each pair of limbs. The lower jaw was removed, and the remaining part then hemisected. One "hemihead" was placed in a moist paper towel, and stored in a refrigerator (12 degrees Centigrade) for later use the same day. Excess bone and muscle was removed from the front and back of the other hemihead, and also skin and muscle from the remaining skull, to further expose the eye. The eye was left

Figure 4. Absorption spectra for the two known photopigments in mudpuppy retina, as a function of stimulus wavelength. Adapted from Fain (1975). A method to equate two two color stimuli for influence on rods and cones is shown. From Frumkes and Eysteinsson (1987)


Mudpuppy
0
-0.5
-1
Log relative sensitivity
C 0.2<R
cone
C 0.75>R
rod
450
500
550
600
650
Wavelength [nm]

attached to the skull, which in turn was placed on a cotton ball soaked in amphibian Ringer solution. After removing the skin surrounding the eye with a relatively course scissors and forceps, all further dissection was performed with the aid of a dissection microscope using visible light. Experience proved that this yielded the most viable preparation. When extreme dark adaptation was desired, the retina was allowed to dark adapt for >10 minutes while the cell was impaled with the recording electrode. Since the estimated "absolute threshold" of second order neurons seemed comparable to human absolute threshold measured psychophysically (Frumkes, personal communication), this procedure was deemed superior to dissections which were performed using inadequate visible light or infrared illumination.

After removing the skin surrounding the eye, all further dissection was performed with a vannas scissors and #5 dumont style forceps. After removing the cornea, the pupil was made wider by cutting small pieces of the iris, and then the lens was pulled out. If not too viscous, most of the vitreous was soaked out after removing the lens. If too much vitreous remains, electrode penetration is difficult, but too much vigor in trying to remove thick vitreous can result in detachment of the retina from the pigment epithelium.

All recordings were made from the superfused eyecup preparation. The eyecup was surrounded with a kimwipe tissue with a hole large enough to expose the retina. The tissue was placed on the eye so that the hole was over the center of the retina. Small cuts were made into the tissue around the hole, so that edges of the tissue could be pushed towards the remainder of the iris. The purpose of the kimwipe tissue was to draw out via capillary action the superfusate, thus achieving faster turnover of solutions. This preparation was placed on a chlorided silver ground electrode. The electrode and preparation were placed in a light tight Faraday cage on a bidirectional slide, and the photic stimuli were aligned and focused upon the retina.

C) Superfusion.

Superfusion was accomplished with a gravity feed apparatus. A separate, electrically grounded Faraday cage was hung over the experimental chamber and contained a cradle with eight 250 ml reservoir bottles. A valve was connected to each tubing, and an 18 guage teflon tubing was connected to the valve. The 8 teflon tubes were wraped together, electrically shielded, and fed into the light tight Faraday cage. The tube from each bottle was connected to a mixing well whose output was brought to the retina through a 1 mm (inner diameter) rubber

tubing and a capillary pipette. The capillary pipette was 1.2 mm in outer diameter and fashioned by heat so that approximately 3 cc of superfusate flowed through it every minute. During experiments, the tip of the pipette was placed at the edge of the eyecup, so that a continuous flow of the superfusate was obtained. The kimwipe tissue covering the upper part of the eyecup was the first stage of a drainage system, followed by a line of another, rolled-up, kimwipe which led to a plastic tubing, which in turn led to a collecting reservoir. When the kimwipe tissues were soaked, the Ringers solution would leak into the plastic tubing. It was imperative to obtain a smooth continuous flow through the drainage, since any dripping would cause serious electrical artifacts. In this way, exchange of fluids occured in less than 30 sec. All the 8 bottles were aerated with 100% oxygen.

One bottle contained the Ringer's solution for the amphibian retina described by Miller and Dacheux (1976). This basic amphibian Ringer's solution consisted of:

111.0 mM NaCl	3.0 mM KCl
1.8 mM CaCl2	1.0 mM MgCl
3.0 mM HEPES (Sigma H-3375)	11.0 mM Glucose

This solution is referred to below as the "control" solution. All other solutions were made by adding small quantities of additional agents to the control solution. All solutions were titrated to a pH of 7.8 with a

solution of 5% NaOH. All pharmacological agents were added to the control Ringer's solution directly, except lead chloride, which was added to boiling distilled water first and stirred until dissolved. The ingredients of the Ringer's solution were then added to the lead chloride.

The dose of each pharmacological agent used was determined on the basis of results published by previous authors, as well as on the basis of considerations relevant ot this study. For D-O-Phosphoserine (DOP), 2.5mM is the lowest dose needed to have any effect in mudpuppy retina (Slaughter and Miller, 1985a), and higher doses have effects on the inner retina directly (Slaughter and Miller, 1986). But in some experiments in the distal retina, 5mM DOP was used in order to get maximum effects on horizontal cells. The same considerations applied to Kynurenic acid, since 2.5mM have been found to be the mininum dose needed for any effect (Miller, Slaughter, Coleman and Massey, 1987). Only one dose of 2-amino-4-phosphonobutyric acid (APB) was used, or 30uM. This is a high dose, but most effective in blocking depolarizing bipolar cells (Slaughter and Miller, 1981). I found that 100uM was the minimum effective dose of lead chloride in mudpuppy, a concentration 10 times the one used by Fox and Sillman (1979) in Rana. Doses higher than 200uM, however, were

less reversable. Since the physiological effect of dopamine in mudpuppy retina was also unknown, I gradually increased its dose until an effect was seen on horizontal cells. I found that a dose of at least 1mM dopamine was needed, which is a factor of 100 higher than in teleost fish (Negishi & Drujan, 1979) or turtle (Piccolino, Neyton and Gerschenfeld, 1984). Doses higher than 1mM were less reversable.

The pH of all solutions were checked before each experimental session, and adjusted if necessary. New solutions were made every week. This was done to avoid changes in Na+ concentrations due to repetitive adjustment of the pH, and to prevent any biological contamination of the superfusates. After each day of work, the bottles were capped with rubber stoppers and stored at 4 degrees Centigrade in a refrigerator.

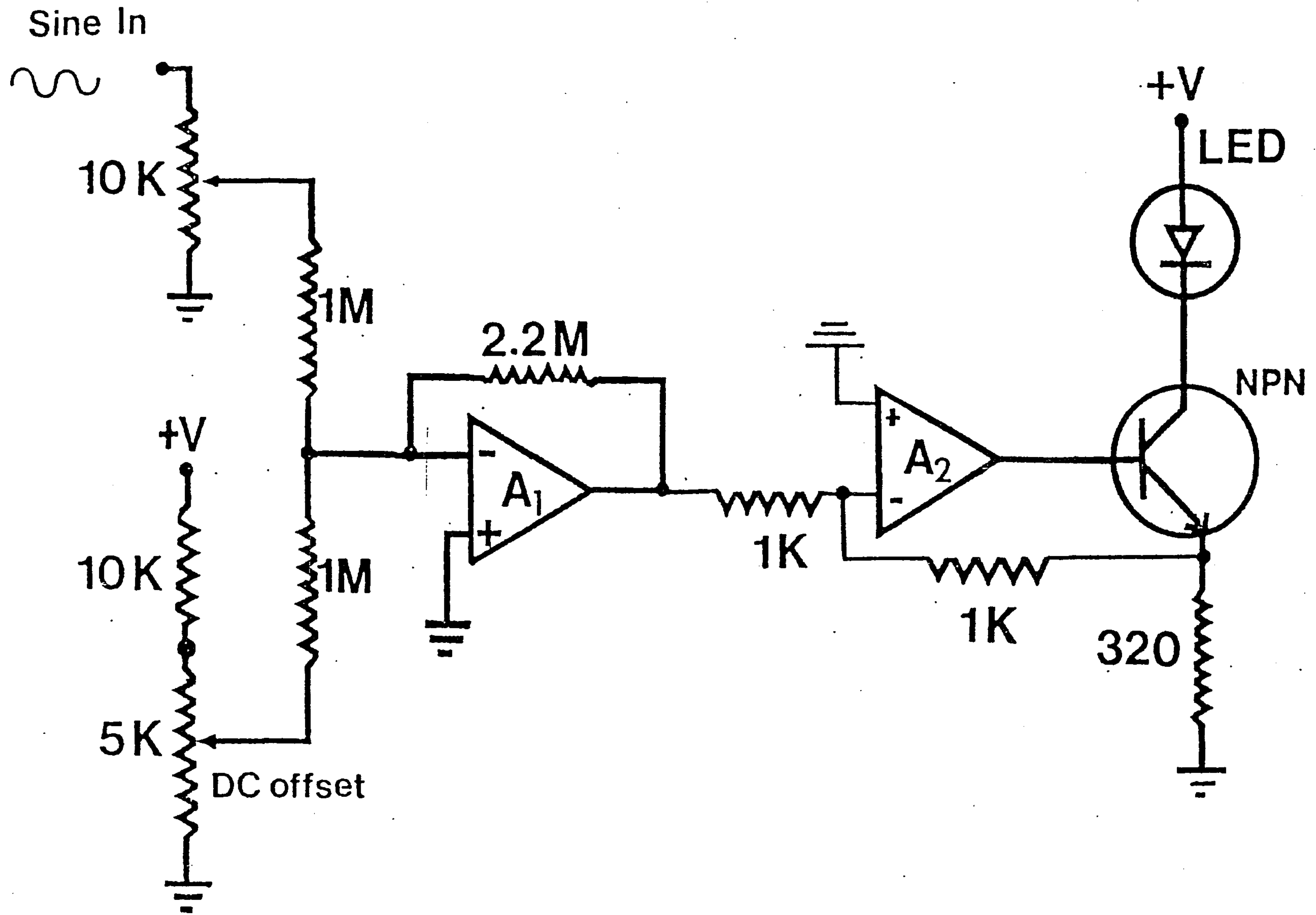
D) Light Stimulation.

All photic stimuli stemmed from a two-channel optical system, with the configuration described previously (Frumkes and Eysteinsson, 1987; Frumkes and Miller, 1979). One of the light sources was a 100 Watt tungsten halogen bulb (FCR). This 12 volt source was intentionally underpowered at 11.5 volts to preserve bulb life. Also, infrared illumination was filtered out of

this light beam. The irradiance and spectral composition of this light source was controlled by means of narrow band interference filters and two, 4-log unit circular neutral density wedges geared in counter rotation to provide 8 log units of intensity adjustment. The stimulus duration of this source was electronically controlled by an electromagnetic shutter (Uniblitz, Vincent Associates).

The other light source was a red light-emitting diode (LED) (H2K, Stanley Electric, Tokyo), with a peak spectral output of ~650nm, and a half-bandwidth of ~40nm. This source provided either a sinusoidally flickering light or rectangular, "on-off" waveforms. In either case, this source was controlled by the circuit shown in figure 5. The 5K potentiometer in the lower left of the figure controled the source intensity of the LED, and was set so that the maximum possible intensity was obtained at 96% depth of modulation of the sinusoidal flicker. Total deviation of the photic output from a sinewave was <2%, as measured by the procedure of Nygaard and Frumkes (1982). Light stimulation was in general monitored with two phototransistors, one attached to the back of the LED, the other facing the neutral density wedges in the tungsten channels. In this way the light intensity of both could be monitored on line during experiments. The depth of modulation of the LED was

Figure 5. Schematic for the circuit controlling the light emitting diode (LED) used as one of the light stimulators in this study. Amplifiers A1 and A2 are one half of a 1458 (dual 741) Op Amp. The NPN transistor is a 2N2222, and +V and -V are outputs of a dual tracking +/-15V power supply. "Sine in" represents input from a waveform generator. The 10K potentiometer controls the depth of modulation, while the 5K potentiometer controls the source intensity. From Frumkes and Eysteinsson (1987).



Sine In
10 K
1M
2.2 M
+V
10 K
1M
A1
1K
A2
+V
LED
NPN
1K
320
5 K
DC offset

controlled by the 10K potentiometer shown on the left of fig. 5. Although the 5K pot permits electronic control of source intensity, the average irradiance of the stimulus stemming from this LED was controlled by means of fixed neutral density filters.

The spatial configuration of the stimulus generated by the tungsten source was either controlled with a fixed circular aperture of 2mm in diameter, or series of circular apertures ranging from 0.2 mm to 3 mm. Since this was the source for the diffuse background, the 2 mm aperture was most frequently used, unless otherwise specified. The other channel provided either series of circular spots ranging from 0.2 mm to 3 mm, or a slit stimulus that was 0.1 mm or wider, which could be either widened or moved across the retina in 50 um steps.

The temporal properties of stimulus were controlled by means of a function generator (B&K Precision, Dynascam Corp.) in case of sinuoidal flicker, and an 8-channel electronic timer (Winston Electronics) for rectangular waveform stimuli.

E) Intracellular Recording.

Intracellular records were obtained with microelectrodes fabricated from "omega dot" glass (Glass Company of America), with a 1.2 mm outer diameter and 0.6

mm inner diameter. The electrodes were pulled with a vertical puller (Narishige Co., Tokyo), filled with 2M potassium acetate and backfilled with 2M KCl. The resistance of the electrodes ranged from 300-500 megohms. The tip of the electrode was dipped into a 2% solution of dichloro-dimethylsilane (Eastman Kodak 9650) in carbon tetrachloride. The purpose of this treatment was to form a hydrophobic coating on the tip, which aids the lipid bilayer that constitutes the membrane of the neuron to maintain an adequate seal around the electrode.

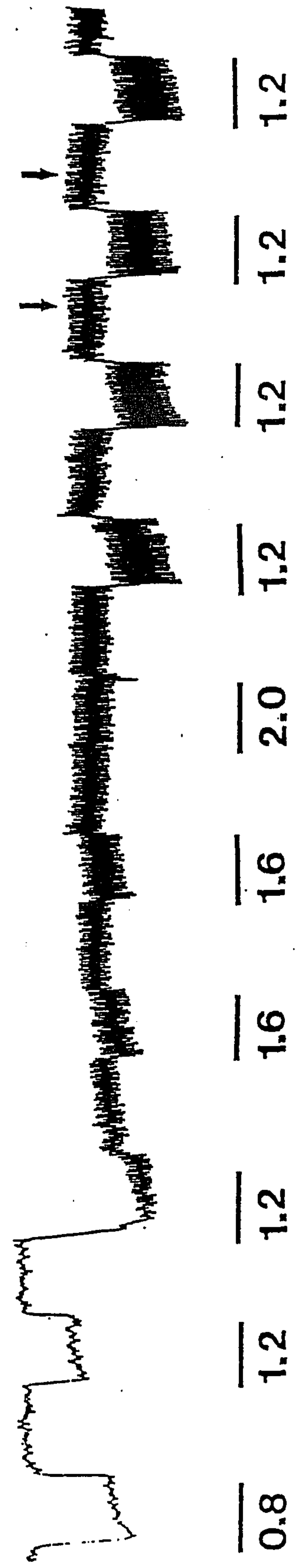
The responses recorded were amplified with a DC coupled high input impedance amplifier with capabilities for current injections (WPI, model 701). The output of the amplifier was fed into a digital voltmeter that provided a continuous measure of the membrane potential, and into one channel of a four-channel FM cassette tape recorder (Vetter C4) with a flat frequency response between 0 and 300 Hz. The output of the amplifier was also fed into a differential offset amplifier with a gain of 10, which allowed for a compensation for the cell's standing membrane potential (bypassing the digital voltmeter), so that the recording could be placed in view on a storage oscilloscope (Textronics 5111). The output of the offset amplifier was fed into another channel of the tape recorder for storage. A third channel on the tape recorder was used to store the stimulus trace, and the fourth channel was used for trigger pulses or output

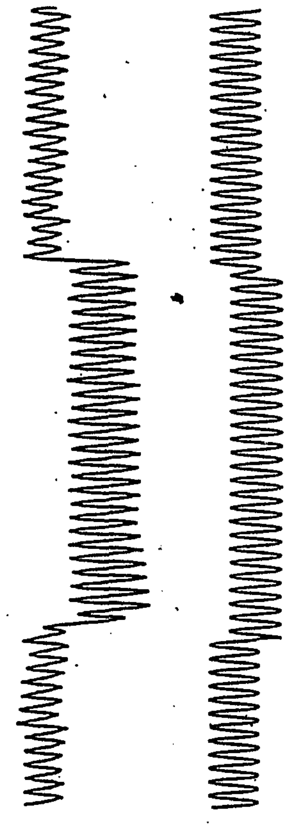
from the current monitor of the intracellular amplifier.

Illustrations of intracellular records were made by feeding back records from the tape to an oscilloscope or rectilinear polygraph, from which they were then photographed. For some records a heat-sensitive polygraph was used (Astro-Med Inc.), which often necessitated retouching the records. For other figures, a pressurized ink polygraph (Brush, Gould Inc.) was used, which produced cleaner records. The heat-sensitive polygraph was used because the Brush polygraph has fewer choices for sweep speed than the Astro-Med, and only two pens, while the heat-sensitive one has four pens.

In a typical experiment, an impaled neuron was identified first on the basis of physiological criteria established by Miller, Frumkes, Slaughter, & Dacheux (1981). The response of the cell to two diffuse 2 sec. stimuli, from either light source, was examined. Subsequently, the response to focal flicker from the red LED and the response to the LED and diffuse background together, were examined. Figure 6, taken from Frumkes & Eysteinsson (1987), shows typical data obtained in the first few minutes of experimentation with a _Xenopus_ horizontal cell. The procedure used in the first minutes of this previously published experiment, was identical to that used during the first minutes of the experiments on the mudpuppy neurons in this study. The neuron shown

Figure 6. Typical experimental procedure used during the first minutes of a recording. The illustrated recording is from a Xenopus horizontal cell. Most cells were also tested for center-surround by continuously illuminating the center with a very high frequency flicker, while intermittently presenting the background. The upper trace shown is a continuous recording at a slow time scale of the polygraph. The first two responses are to the background stimulus at two different intensities. This 500nm light provided a quantal flux of log quanta cm-2 sec-1 of 11.20, if unattenuated by neutral density filters. The short horizontal bars under the response indicates presentation of this stimulus, and the numbers under the bars show the log attenuation in each case. The sinusoidallly flickering stimulus was continually presented during the period shown by the long horizontal bar. It was 9hz, and 630um in diameter, and provided quantal flux of log quanta cm-2 sec-1 of 9.04 if unattenuated by filters. From Frumkes and Eysteinsson (1987)


10 mV
5 S
0.8
1.2
1.2
1.6
1.6
2.0
1.2
1.2
1.2
1.2

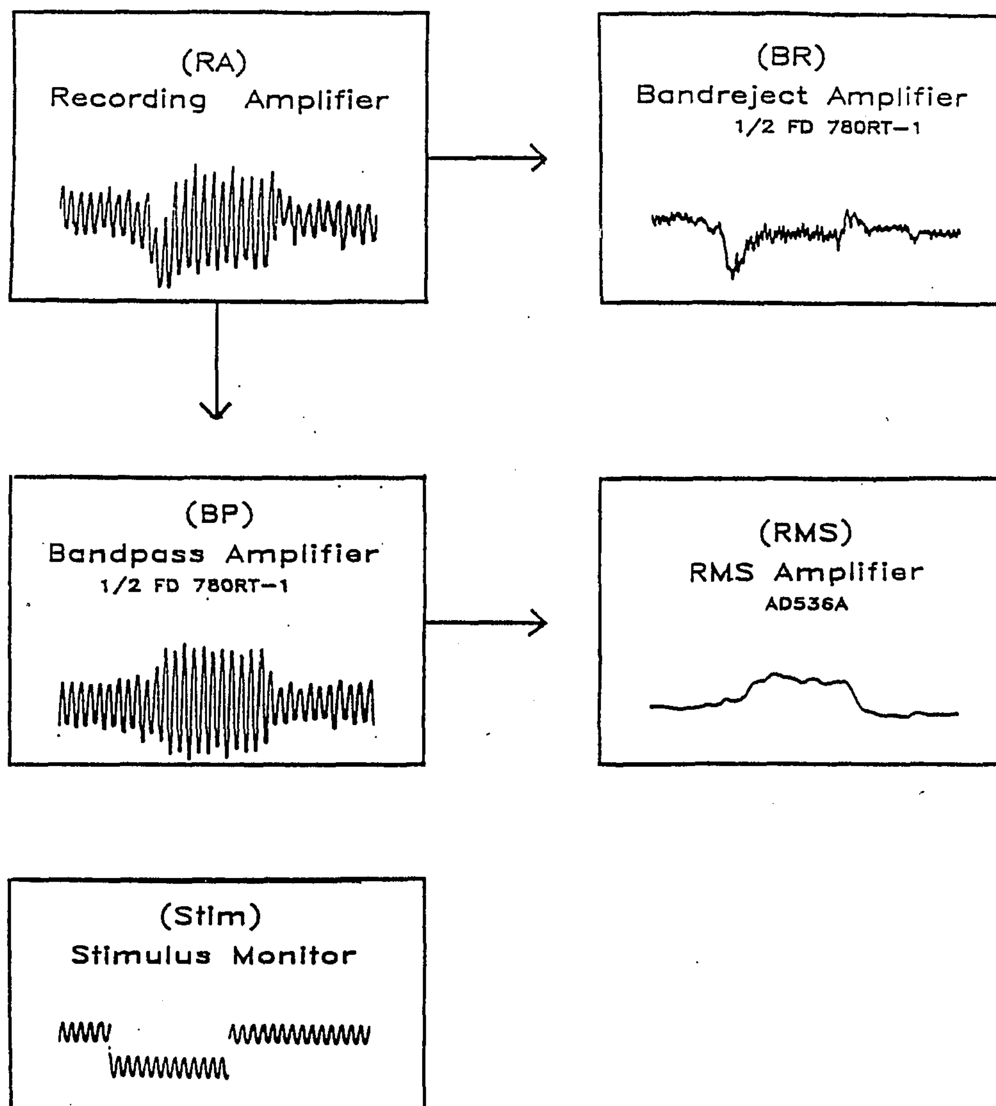

10 mV
1 S

was impaled about 15 min after placing the preparation in the recording chamber. The upper record shows continuous data, played back at one polygraph time scale, and was obtained about 1 min after encountering the neuron. The responses shown first are in response to spatially diffuse stimuli of 500 nm in wavelenght and 3 sec. in duration, but with two different levels of irradiance, as indicated. Then a flickering stimulus of 625um in diameter and 9 Hz in frequency, centered with respect to the recording electrode, was presented continuously while diffuse stimuli of various irradiance levels were still presented periodically. The flickering stimulus had a quantal flux of log quanta incident cm-2 sec-1 of 9.04 . The mean potential of the neuron in the presence of continuous flicker but without diffuse background stabilizes within few seconds around -35mV. The time period marked by two arrows is shown again in the lower record with the same voltage gain, but at an expanded time scale. This lower record shows that the response to the flicker is sinusoidal. The response to flicker has a peak-to-peak amplitude of about 7 mV without the background, but shortly after presenting the background, the cell hyperpolarizes and the peak-to-peak amplitude of flicker increases to about 12 mV. The amplitude of flicker under these two conditions was used as an index of flicker sensitivity. In the present experiments in mudpuppy, this type of procedure would then be used to

study the photic response of this neuron in the presence of various pharmacological agents. After identification of each cell and if SRCI was found as shown in figure 6, the suprefusate was switched from control Ringer's to the pharmacological agent chosen as appropriate among those 7 available in each experimental session. The choice of agent, and the dose, was made on the basis of previously published results and the considerations discussed previously in this chapter. The duration of the superfusion of the pharmacological agent was measured, and was determined by the time deemed needed to obtain a physiological effect. The superfusate was then switched back to the control Ringer's solution and remained until the drug effect reversed. After full recovery, another dose or another agent was applied in the same fashion, until all appropriate agents and doses had been tested, or the cell's response to light deteriorated.

In order to highlight some specific effects of the pharmacological agents, taped records were fed through analog filters, as depicted in figure 7. The figure shows a typical analysis of a depolarizing bipolar cell (DPBC), using this approach. The output of the recording amplifier is fed into a matched bandpass-bandreject amplifier. The output from these for the DPBC is shown in figure 7 on the right and below the recording amplifier output. Under the stimulus conditions used,

Figure 7. Schematic diagram of active filters used for data analysis. All recordings are from a depolarizing bipolar cell. The analog filter (FD 78ORT-1) had a bandreject and a bandpass output. The output from the stimulus monitor was used to tune the bandpass/bandreject ratio to the proper response frequency. The output from from the bandpass amplifier was fed to an root mean square (RMS) amplifier (AD536A). The output from each "box" was fed into one polygraph channel and played simultaneously.



(RA)
Recording Amplifier
(BR)
Bandreject Amplifier
1/2 FD 780RT-1
(BP)
Bandpass Amplifier
1/2 FD 780RT-1
(RMS)
RMS Amplifier
AD536A
(Stim)
Stimulus Monitor

the bandreject output provides an index of the receptive field surround mechanism, as well as a measure which can be used to indicate the long term influence of the drug on the ambient membrane potential of the cell. The bandpass amplifier output shows the response to flicker and how flicker response amplitude is influenced by the diffuse background. The output ot the bandpass amplifier was also fed into a root mean square (RMS) amplifier, whose DC level is directly proportional to the peak to peak amplitude of its sinusoidal input, and thus aid in quantification. This is show on the bottom right for the DPBC. The abbreviations used in figure 6 are again used in some of the later figures.

Chapter III: Results.

Part I.

A) The Influence of Lead upon SRCI

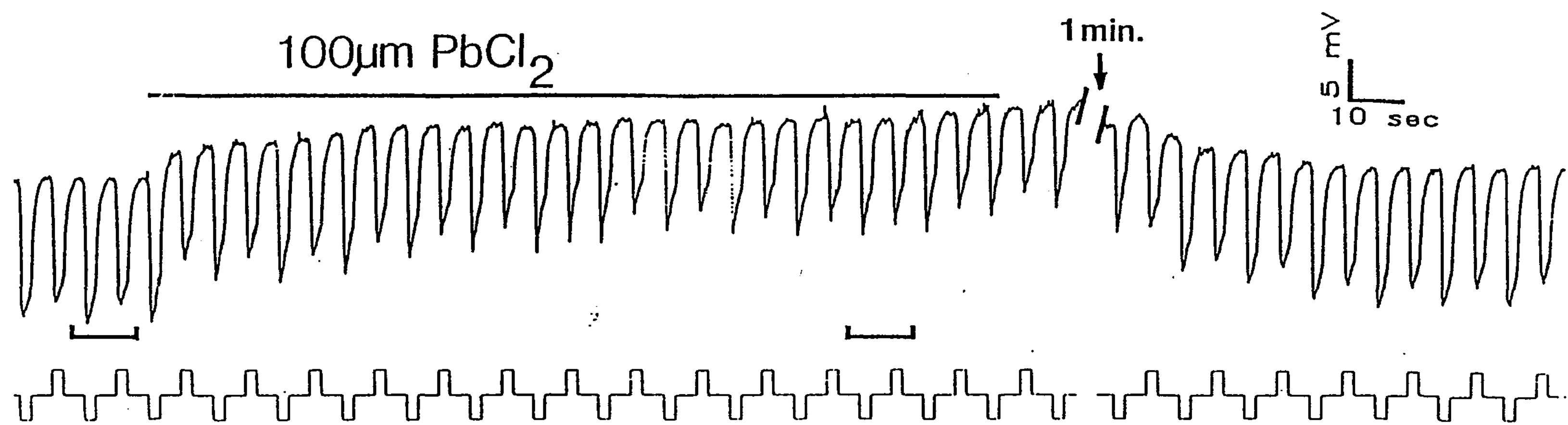
1. Rod input to Horizontal Cells

According to the model in figure 3, SRCI reflects rod modulation of horizontal cell-mediated inhibitory feedback onto cones. If this model is correct, a treatment which selectively interferes with the rod input to horizontal cells without interfering with cone-driven responses should block background enhancement of flicker.

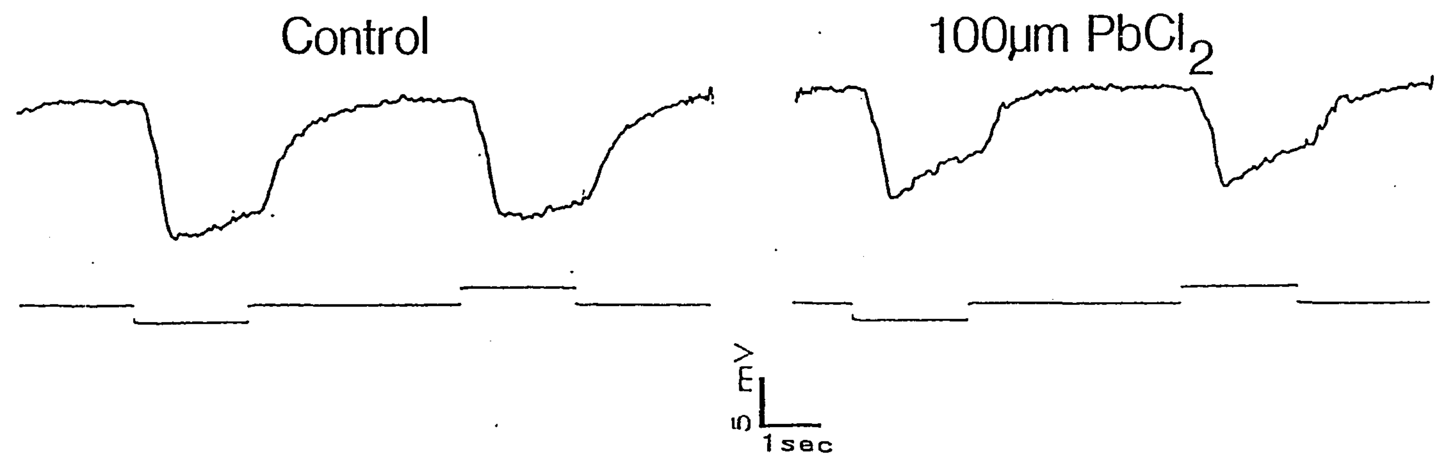
Fox & Sillman (1979) showed that the rod-driven b-wave of the frog ERG can be attenuated selectively by applying heavy metals such as mercury and lead. Since the ERG b-wave reflects post-receptor activity (Brown and Wiesel, 1961), these results indicate that lead reduces rod input into second-order neurons.

If in fact, lead selectively blocks rod-driven horizontal cell response components in mudpuppy, it would be a good agent to test the validity of the model in fig. 3. This proved to be the case. The upper records in fig. 8 show responses of a mudpuppy horizontal cell to diffuse light flashes stemming from a 640 nm (upward

Figure 8. Intracellular recordings from mudpuppy horizontal cell. The upper record is a continuous record from the cell in response to two diffuse stimuli that were alternately presented. One of these stemmed from a 640nm light source and is indicated by an upward deflection on the stimulus monitor, while the other stemmed from a 520nm stimulus, indicated by a downward deflection. These stimuli were equated in intensity for their influence on cones according to the method shown in figure 4, so that the 520nm stimulus had a 0.8 log unit greater influence on rods than cones. The same configuration of the stimulus monitor was used for the lower records, which are the records indicated by brackets in the upper trace, but presented at a faster polygraph speed (note different time calibrations). Lead chloride (100um) was superfused during the time period under the vertical bar in the upper record.



100µm PbCl2
1 min.
5 mV
10 sec



Control
100µm PbCl2
5 mV
1 sec

stimulus deflection) and 520 nm (downward deflection) light flashes. Since the two stimuli were equated for their influence upon cones, the 520 nm stimulus had a 0.8 log unit greater influence upon rods (e.g., see fig. 4 earlier). Hence, as can be seen in the initial control records in the upper left, the 520 nm stimulus produces a measurably larger response. After obtaining several control records, 100 um lead chloride was introduced into the superfusate as indicated by the horizontal bar over the response tracings. The initial upward shift in the records occuring shortly after lead application is probably an experimental artifact: in similar experiments in other horizontal cells, lead chloride more commonly produced an initial slow depolarization, then a hyperpolarization of dark membrane potential. Of greater interest than these slow changes in baseline voltage are the changes in response amplitude. Notice that after 2 minutes in lead, the response to both stimuli becomes more similar in amplitude. To illustrate the overall change in waveform resulting from lead chloride, the pairs of responses marked by brackets on top are reproduced at a faster timescale on the bottom. Under control circumstances, note the slow repolarization of this neuron following offset of either wavelength light flash, a response features designated the <u>rod after potential</u> which is only observed in cells demonstrating rod input (Steinberg 1969). The records in the lower

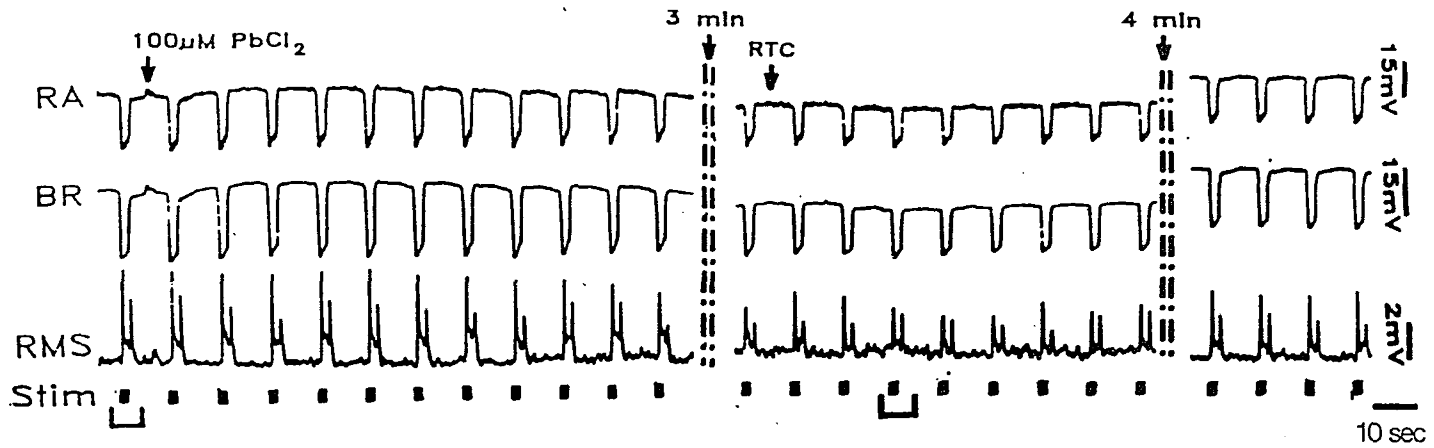
right show that in the presence of lead, the rod after potential is markedly reduced in amplitude. Lead's effects are reversable as shown in the upper right.

Experiments similar to those illustrated in fig. 8 were performed in 8 other horizontal cells. Although the influence upon the dark resting potential was quite variable, lead similarly attenuated rod related responses in 6 of them: in the other 2, the effects of lead were unclear due to response instability. Since I did not perform similar experiments in recording from other cell types, I cannot comment on the underlying mechanism. Collectively, however, these results are sufficiently clear cut to establish that 100 um lead is an appropriate agent to test the model of fig. 3.

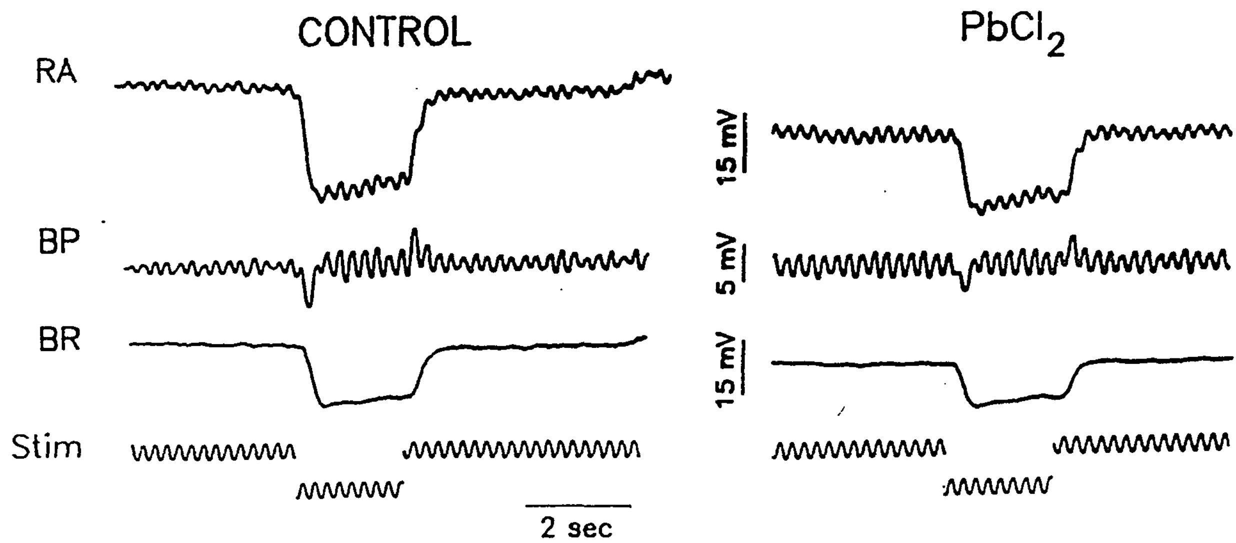
2. Lead Blocks SRCI in Horizontal Cells

Figure 9 shows intracellular recordings from a mudpuppy horizontal cell. In these experiments, a 510 um diameter flickering stimulus of 4.5 hz and 650nm in wavelenght was continuously presented and centered around the recording electrode. In addition, a dim, spatially diffuse background field of 520 nm wavelength was presented at 12 second intervals. The upper portion of the figure shows a record at a slow sweep speed. The traces labeled RA, BR, and RMS represent the output of the recording, bandreject, and root mean square amplifiers respectively. The time of lead application,

Figure 9. Recordings from a mudpuppy horizontal cell, showing the effect of lead on SRCI. The four upper traces represents a continuous record from the cell at a slow polygraph sweep speed. Abbreviations as before, except that "stim" represents presentations of the 520nm background only. A 5Hz small red flickering stimulus was presented continuously. The lower two panels show two presentations of the background, indicated by brackets under the "stim" trace, reproduced at a faster polygraph sweep speed. Note different time calibrations for the upper and lower parts of the figure, and different amplitude calibrations for each output.



100µM PbCl$_2$
3 min
RTC
4 min
RA
BR
RMS
Stim
15mV
15mV
2mV
10 sec



CONTROL
PbCl$_2$
RA
BP
BR
Stim
15 mV
5 mV
15 mV
2 sec

as well as gaps in the record are clearly indicated. Although this slow timescale has a number of advantages, it precludes illustration of the flicker following response. For this reason, the two lower panels show at an expanded time scale, the time periods marked on top by the brackets: BP indicates the bandpass output records at an expanded time scale. These lower records were obtained just before lead application (labeled "control") or just after the removal of lead (labeled "$PbCl_2$").

As seen most clearly by the bandpass record in the lower right, SRCI is quite evident prior to lead application: as seen most clearly in the BP record on the bottom, the peak to peak amplitude of the flicker following response increases from 1.5 mV in the dark to 3.5 mV in the presence of the background. As seen on top, lead application causes the cell to initially depolarize slightly and then hyperpolarize by approximately 8 mV. Lead was removed after about 5.5 minutes of application, e.g., at the time marked by the arrow labeled "RTC" (return to control). As seen most clearly in the BP record on the bottom, shortly after the removal of lead, the flicker response amplitude is barely influenced by presenting the dim background field, and flicker amplitude in the dark (approximately 3 mV) is similar to the amplitude observed under control circumstances when the background is present. To the

extent that lead acts by selectively reducing rod input to horizontal cells (as well as perhaps other second order neurons), these results are consistent with the model of fig. 3. After 5 minutes in control Ringers the uppermost BR record shows considerable recovery in the response to the diffuse stimulus, and the RMS record shows almost complete recovery of SRCI. As might be expected from this action as well as the results in fig. 8, the response to the diffuse light (most clearly seen in the BR records) is also reduced by lead. Although the influence of lead upon the ambient membrane potential varied somewhat from cell to cell, similar results upon flicker were observed in the other 6 horizontal cells so studied.

Other heavy metals, such as cobalt and zinc, have been reported to have a similar selective influence upon rod activity (Evans, Hood & Holtzman, 1978). For this reason, several experiments similar to those represented by figs. 8 and 9 were then performed with these ions. In the concentrations examined, all of these ions were found to block all horizontal cell activity and hence, were inappropriate for use in the present study.

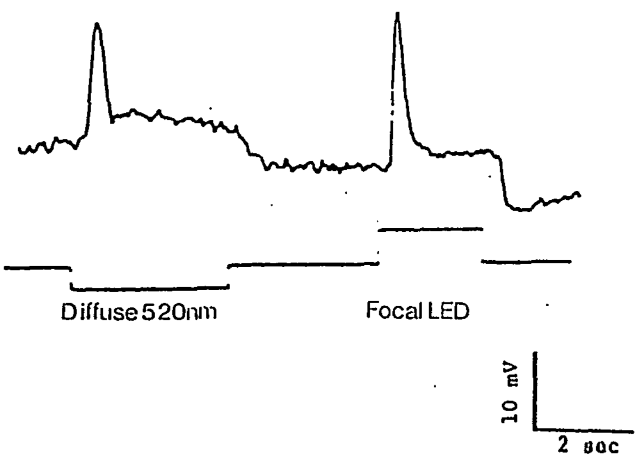
B) The Role of Horizontal Cells in SRCI: Bipolar Cell Recordings.

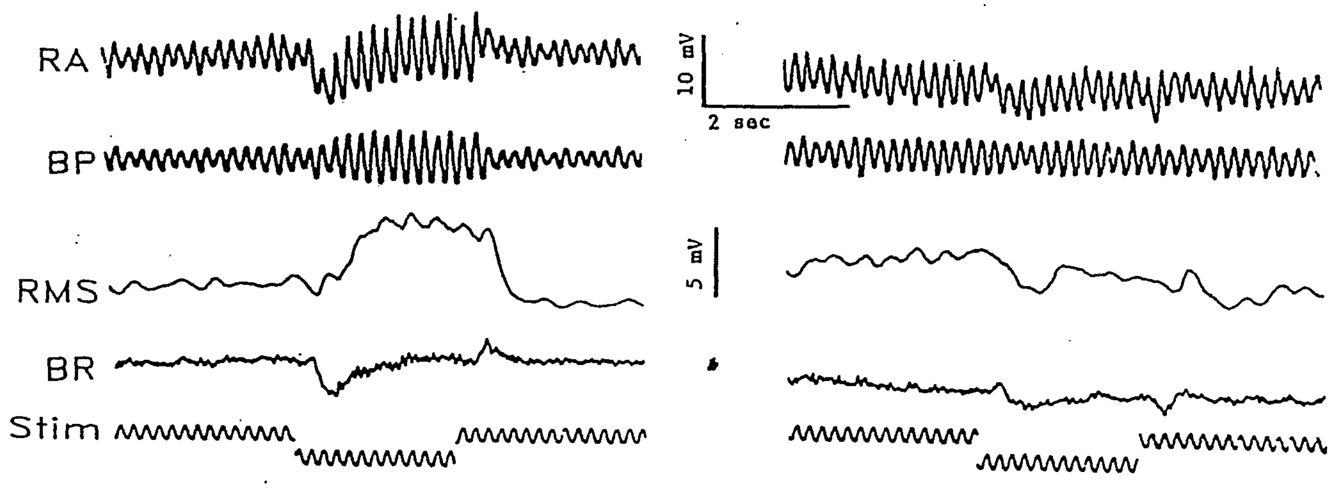
According to the model in figure 3, any agent which blocks the horizontal cell response to light should block SRCI in all other types of neurons. By duplicating the effects of selective rod-light adaptation, such agents should enhance flicker to levels normally only seen in the presence of of a background field. As indicated in the introduction, both kynurenic acid (KyA) and piperidine dicarboxylic acid (PDA) block light responses in horizontal cells and hyperpolarizing bipolar cells: although a less potent blocker, D-O-Phosphoserine (DOP) partially blocks the horizontal cell responses and has the advantage of not influencing the hyperpolarizing bipolar cell (Slaugther & Miller, 1985). It should be stressed that all of these effects have been well documented in the mudpuppy, the species used in the present study. In the course the present study, I additionally confirmed these results using our usual SRCI paradigm. As exemplified by the data presented in figs. 23-25 in the appendix, all three of these agents block all aspects of horizontal cell activity including the response to flicker in the dark, the response to flicker in the light, as well as the response to a diffuse light flash. For this reason, these agents can not be used with horizontal cell recordings to test the validity of

the model in fig. 3. They are, however, quite appropriate for testing the model when recording from other types of neurons.

Figure 10 illustrates a typical SRCI experiment using Kynurenic acid (KyA). The data in the upper left show responses to spatially focal and diffuse stimuli and indicate the cell type as a depolarizing bipolar cell. The lower data show a SRCI experiment using the paradigm outlined in fig. 9 above. The two panels indicate the influence of a dim background field upon the response to focal flicker under control conditions (on the left) and in the presence of the drug, in this case 2.5 mM KyA. The five traces represent the output of the recording, bandpass, RMS and bandreject amplifier and of the stimulus monitor (labeled RA, BP, RMS, BR, and Stim. respectively). Under control circumstances, the background clearly enhances flicker as seen most clearly in the BP and RMS records, and the antagonistic receptive field surround of this cell is quite evident, as seem most clearly in the BR record. Shortly after obtaining this control record, KyA was introduced. The record shown on the right was recorded after 1 minute exposure to KyA. As seen most clearly in the BP and RMS records, flicker in the dark is greater than observed under control circumstances, and background enhancement of flicker is absent. In addition, the BR record shows that this

Figure 10. Intracellular recordings from a depolarizing bipolar cell in mudpuppy retina. The responses at the top of the figure are to diffuse 520nm stimulus and a focal LED. Note separate calibration markers for these below the stimulus trace. The left panel shows records from this neuron in response to a continuous focal red flickering stimulus and one presentation of rod-adapting background, while superfusing the retina with control Ringer's solution. The right panel shows recordings taken after 1 minute superfusion with 2.5mM kynurenic acid. Note separate calibration marker for the amplitude of the RMS records. Abbreviations stand for the same as in previous figures.

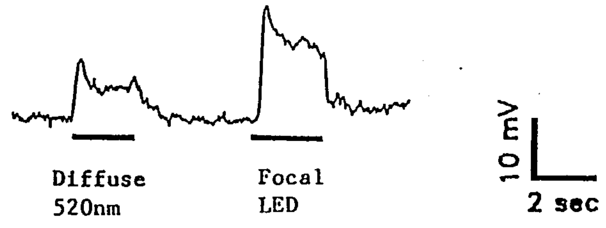

Diffuse 520nm
Focal LED
10 mV
2 sec


Control
1 min in 2.5mM KyA
RA
BP
RMS
BR
Stim
10 mV
2 sec
5 mV

treatment reduced but does not completely abolish the antagonistic surround of this cell. Although not illustrated, KyA's effects reverse within 1 minute after removal from this cell. Although not as effective, figure 11 shows results from a similar experiment involving a recording from another depolarizing bipolar cell in which DOP rather than KyA was used to partially block the horizontal cell photic response. Although somewhat less effective, DOP has qualitatively similar effects.

Since DOP has no direct action upon any bipolar cell (Slaughter and Miller, 1985), it was used to assess the validity of the model in fig. 3 while recording from hyperpolarizing bipolar cells. Figure 12 shows such an experiment. The records in the upper left in response to a small spot of light and a diffuse light of two seconds duration clearly identify the cell type. The experiment illustrated on the bottom shows the influence of DOP upon SRCI. The influence of DOP upon this cell is similar to that of DOP and KyA upon depolarizing bipolar cells as shown in fig. 10 and 11 above, save that in this particular cell, DOP has no obvious effect upon the receptive field surround mechanism as shown in the BR record. In some other hyperpolarizing bipolars, however, the surround mechanism was somewhat attenuated by DOP application.

Figure 11. Intracelluar recordings from a mudpuppy depolarizing bipolar cell. The two responses at the top of the figure are to a diffuse 520nm stimulus and focal red LED. Note the separate calibration markers for these responses on the right. The left lower panel shows recordings from the cell taken in control Ringer's, in response to a continuous focal red flicker and one 3 seconds presentation of the 520nm background. The right panel shows recordings taken after 1 minute superfusion with 5mM D-O-Phosphoserine (DOP). Abbreviations as before.



Control

1 min in 5mM DOP

RA

BP

10 mV

RMS

10 mV

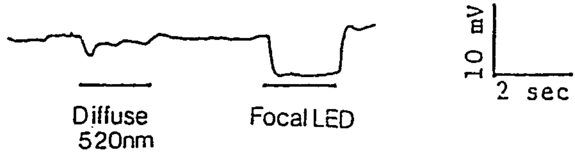
BR

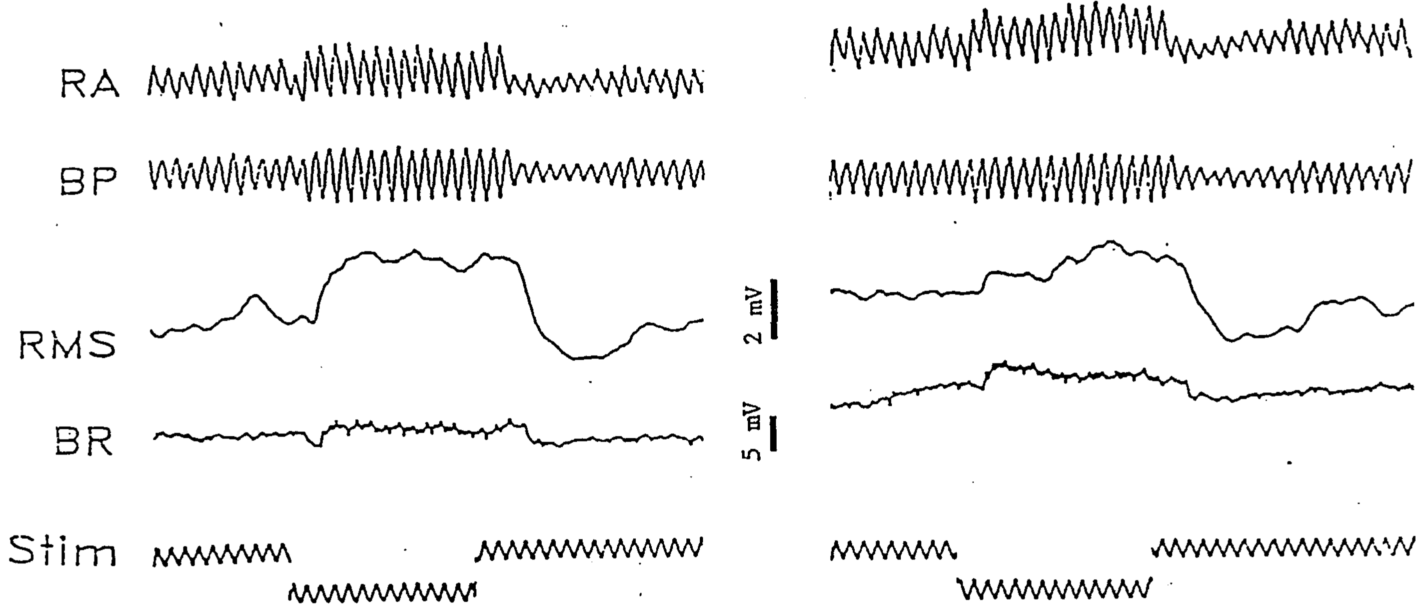
10 mV

Stim

10 mV
1 sec

Figure 12. Intracellular recordings from a mudpuppy hyperpolarizing bipolar cell. The top row shows responses to the diffuse 520nm stimulus and a focal red (LED) stimulus. Note separate calibration markers for these responses. The left panel of records shows one recording in control Ringer's solution in response to a continuous focal flicker and one presentation of the 520nm background. The right panel shows similar records after a 2 minutes superfusion of 2.5mM D-O-Phosphoserine (DOP). The calibration marker applies to all records in both panels, except the amplitude of the root-mean square (RMS) records. Note the calibration marker, showing 2mV, for the RMS records. All abbreviations the same as before.


Diffuse
520nm
Focal LED
10 mV
2 sec


Control
2 min in 2.5mM DOP
RA
BP
RMS
BR
Stim
2 mV
5 mV
10 mV
1 sec

C) Recordings from Cones.

1. The Spectral Characteristics of the Antagonist Surround of Mudpuppy Cones.

In the course of the present study, I recorded from 8 different cones: in only 3 were responses sufficiently stable to perform any pharmacological experiments. The data in figs 13 through 16 were all obtained from the same unit which remained reasonably stable for over one hour. Before obtaining any of the illustrated data, I had in the same penetration, first recorded from a horizontal cell. After penetrating approximately 30 um further into the retina, I obtained this recording and noted that the responses of this cell to a small spot and diffuse light of the same irradiance were quite similar. This is not the case in the most similar response from another neuronal type, the hyperpolarizing bipolar cell (see Frumkes and Eysteinsson, 1987, fig. 11 ; see also fig. 12 in the present study). Figure 13 shows a continuous recording from this cell approximately two minutes after intial penetration. The first three illustrated responses are to 2 second flashes that were either 430 um diameter and stemmed from the LED, or were diffuse and 520 nm in wavelength. After obtaining three responses, the LED was then turned on continuously as

Figure 13. Continuous intracellular recordings from a mudpuppy cone. The first three responses are to 2 second flashes that were either diffuse, 520nm stimuli, indicated by the upper row of stimulus traces, or 430um stimuli, stemming from the LED, as indicted by the lower row of stimulus traces. The values below the stimulus traces indicates the amount of attenuation provided by neutral density wedges for the 520nm stimuli. The LED was turned on continuously at the point indicated by the dot attached to the arrow. The two following stimuli were presentations of the diffuse 520nm field, in order to test for antagonistic surround. The two lower rows of records are a continuation of the first, during which the LED was continuously on, while the wavelength of the diffuse field was 640nm in the second row, and 520nm in the third row. Note changes in attenuation of the diffuse stimuli, as expressed in log units of attenuation under the horizontal bars.

5 mV
2 sec
520nm
LED
−2.4
−2.4
−2.4
−2.4

640nm
−4.0
−3.2
−2.8
−2.4
−2.0
−1.6
−1.2

520nm
−3.2
−2.6
−2.4
−2.0
−1.6
−1.2
−0.8

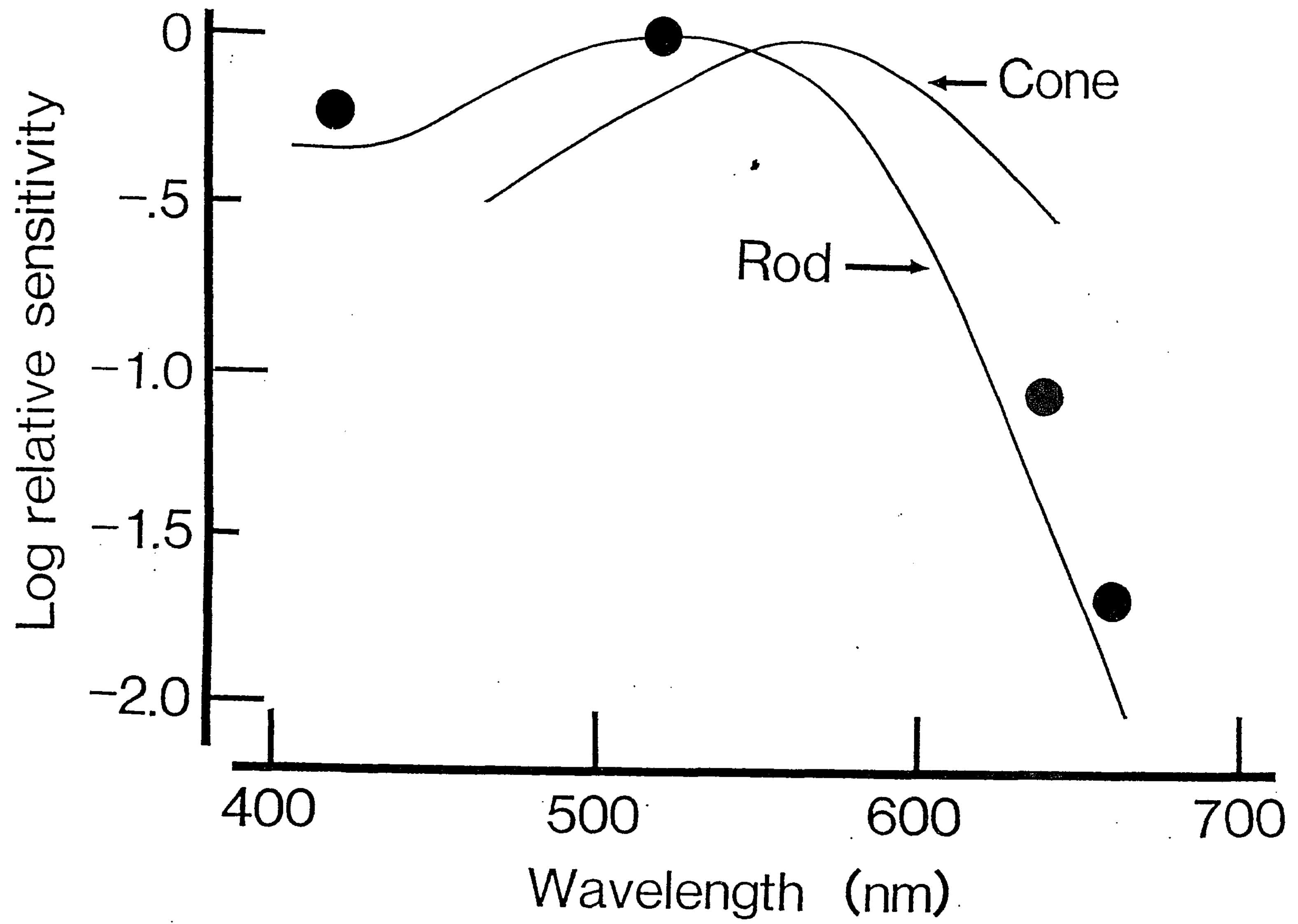
indicated by the dot attached to the arrow. Then the diffuse 520nm stimulus was presented twice in order to test for the presence of an antagonistic surround. The presentation of these stimuli is shown by horizontal bars, and the values below the bars indicate the attenuation provided by neutral density wedges. The antagonistic surround of this cell is evidenced by the depolarization elicited by this diffuse stimulus. The second and third rows are a continuation of this recording, during which the focal LED stimulus remained on. For the records illustrated in the second row, I examined the influence of 640 nm diffuse lights of varying irradiance. Notice that as the attentuation is decreased from log -4 to log -2, the depolarization produced by this diffuse light increases. A slightly supraoptimal stimulus intensity caused a decrease in this depolarizing response component; a further increase caused a considerably change in response waveform. The third row shows a similar experiment immediately afterwards, but using a 520nm diffuse stimulus. Similar data were then obtained using 420 and 660 nm diffuse light flashes of varying irradiance (unillustrated).

From experiments such as those illustrated in fig. 13, I plotted V log I (depolarizing response amplitude as a function of stimulus irradiance) functions for all four diffuse stimulus wavelengths used (420, 520, 640, and 660

nm). From these data, I generated action spectra (irradiance necessary to produce the criterion response as a function of stimulus wavelength) using criterion voltage response amplitudes of between 2 and 6 mV in 0.5 mV steps. All of these were quite similar in shape. Figure 14 plots those obtained using a 5.5 mV response criterion. In fig 14, log relative sensitivity is plotted as function of stimulus wavelength. The spectral sensitivity curve for mudpuppy rods was taken from microspectrophotometric data published by MacNichol (1986), while the curve for the cones is taken from Fain (1975). The four data points represent the log of the reciprocals of the irradiance necessary to produce the criterion response depolarization. Finally, the vertical position of the two microspectrophotometric curves and the set of data points was shifted vertically so that their minima correspond. This was the only curve fitting manipulation used.

In essence, figure 14 represent the action spectra of the receptive field surround of a mudpuppy cone. It is quite obvious that the receptive field surround of this unit describes a rod action spectrum. This proved to be the case in three other units so examined. It is possible that had similar experiments been performed using an annulus rather than a diffuse light to elicit the antagonistic surround, the action spectra might have

Figure 14. Action spectra for the antagonistic surround of the cone in fig.13. Relative sensitivity was found by measuring the irradiance needed to produce a criterion response amplitude for four wavelengths. The criterion amplitudes used were between 2 and 6mV in 0.5mV steps, which produced functions that were very similar in shape to the function presented in this figure. This figure presents values obtained using 5.5 mV as a criterion response. The spectral sensitivity curve for mudpuppy rods was adopted from microspectrophotometric data published by MacNichol (1986).



0
-.5
-1.0
-1.5
-2.0
Log relative sensitivity
Cone
Rod
400
500
600
700
Wavelength (nm)

revealed some cone-input and therefore be more mesopic in quality. But these experiments clearly show that under our experimental conditions, mudpuppy cones have a strong antagonistic surround dominated by rod-input.

2. Cone-surrounds are mediated by horizontal cell Input.

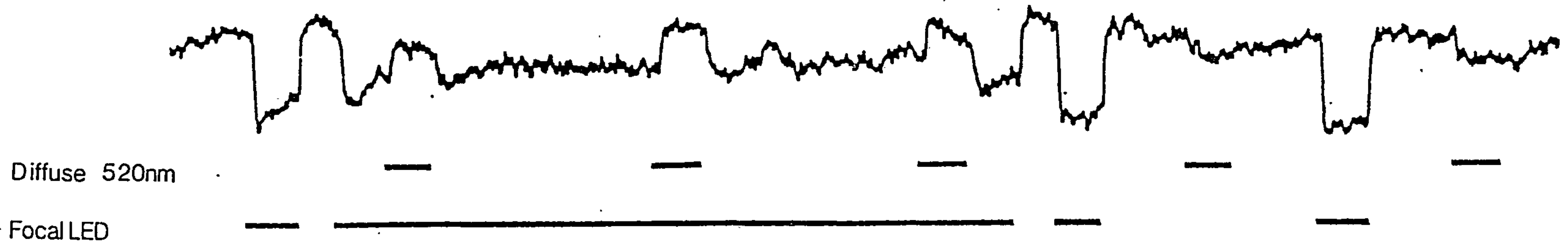
After obtaining the spectral data illustrated in figs. 13 and 14, I then performed the pharmacological experiments shown in figs. 15 and 16 with the same cell. The top row of records in fig. 15 again illustrates the prominent antagonistic surround of this cell using a similar procedure to that illustrated in fig. 13. Notice that when the receptive field center is not adapted, both diffuse and focal stimuli produce a hyperpolarizing response but the response to the diffuse stimulus is much smaller. Also, when the center is adapted, the diffuse light elicits a depolarization. Then 5 mM KyA was introduced to the retina for 2 minutes and I obtained the recording illustrated on the bottom. I used this higher dose (5mM rather than 2.5mM as previously) of KyA in order to obtain maximum effect on horizontal cells, as well as other second and third order neurons. There are two key differences in these KyA data from the control data. First, when the center is adapted, the diffuse stimulus elicits no hyperpolarization. Second, when the

Figure 15. Intracellular recording from the same mudpuppy cone as the previous figure, showing feedback. The upper record, taken in control Ringer's, shows responses to a diffuse 520nm stimulus (upper row of vertical bars) and to a focal (250um) red stimulus generated by the LED (lower row of bars). Note the duration of the red stimulus. The lower row of records shows responses to the same stimulus paradigm, but after a 2 minutes application of 5mM kynurenic acid. Calibration is the same for both records.

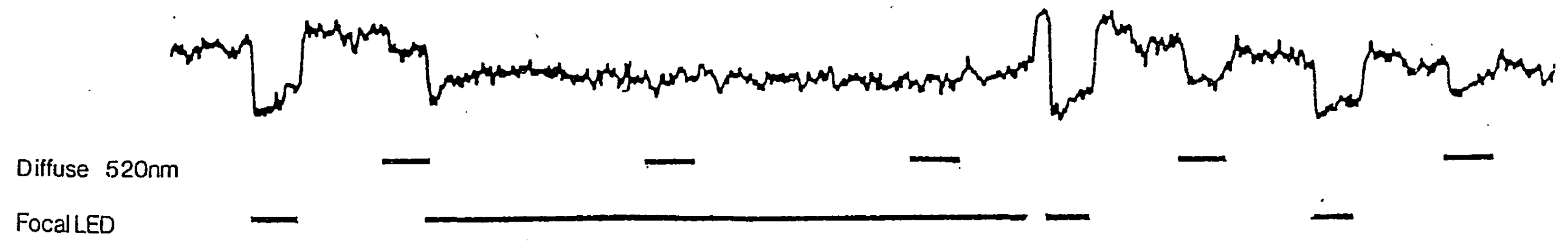
10 mV

2 sec

Control



2 min in 5mM KyA

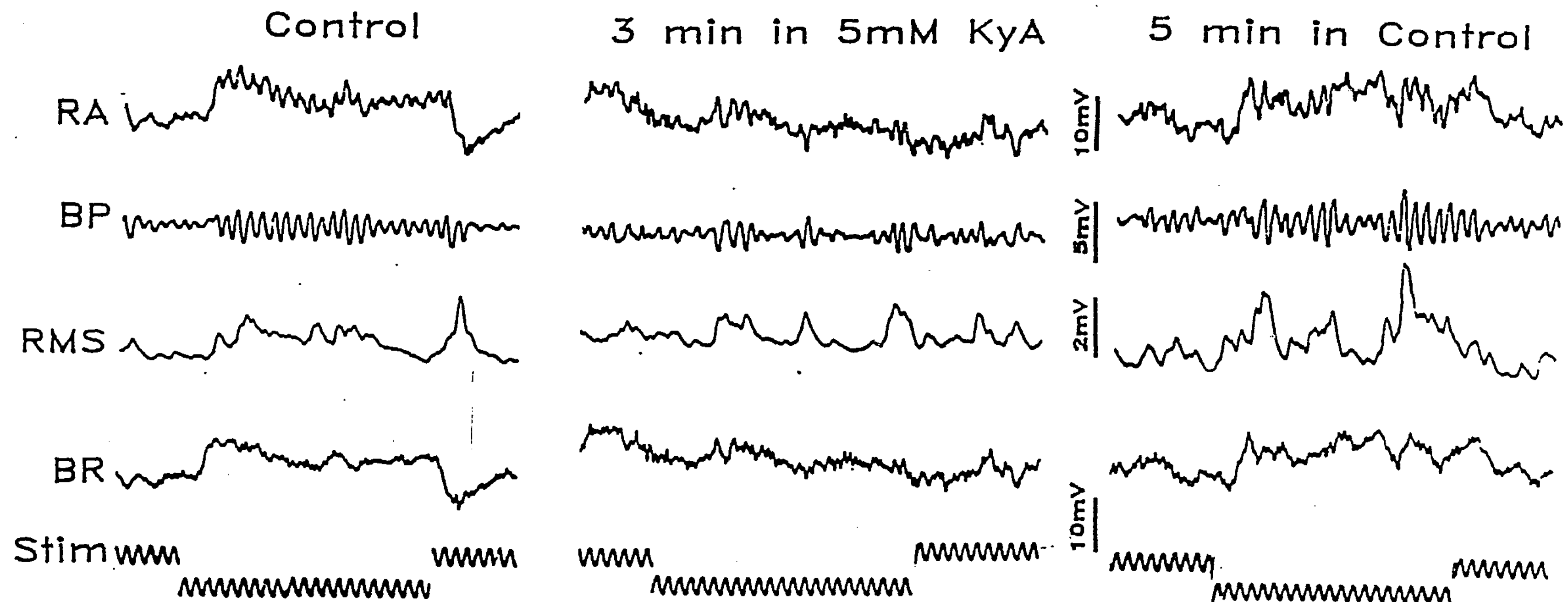


center is unadapted, the diffuse stimulus elicits a much larger response than for corresponding data obtained under control circumstances. Both these observations clearly indicate that KyA blocked the surround mechanism of this cone. I interpret these data in the following way. By suppressing all horizontal cell activity, the horizontal cell mediated receptive field surround of cones is removed. All of KyA's effects proved reversible upon return to control Ringer's.

3. KyA blocks SRCI in Mudpuppy Cones

After obtaining the bottom records in fig. 15, control Ringer's was introduced for about 10 minutes and I then proceeded to study SRCI in this neuron. Illustrative data are presented in fig. 16. These have the same format as illustrated for the bipolar cell in fig. 10, save that the three panels illustrate initial control data, responses in the presence of KyA, and data obtained after return to control (the left, middle, and right panels respectively). Although the flicker following response of this cell was never ideal, the intial control record clearly shows evidence for SRCI (most obviously in the BP record) and the antagonistic surround of this cell (in the BR record). The middle panel shows a record taken after 3 minutes in kynurenic acid. Although somewhat intermittent, flicker following

Figure 16. Recordings from the same cone as in figure 15, showing the effect of kynurenic acid on SRCI. Abbreviations the same as before. Same time scale for all traces, but note different amplitude calibration for each output. The leftmost panel shows recording in control Ringer's solution in response to a continuous red flickering stimulus (7Hz), and 520nm background. The middle panel shows a recording after 3 minutes application of 5mM kynurenic acid, and the rightmost panel shows a recording 5 minutes after returning to control Ringer's solution.



Control
3 min in 5mM KyA
5 min in Control
RA
BP
RMS
BR
Stim
10mV
5mV
2mV
10mV
1 sec

is still obvious in this cell as seen most clearly in the BP record, but bears no relationship to the presence or absence of the background field. Also, the BR record shows no evidence of the surround of this cell. The right-most panel shows the last recordings obtained before losing this cell, approximately five minutes after returning to control Ringer's solution. Although recovery is not yet complete and although the response is quite unstable, most of kyA's effects had reversed. I lost the cell shortly after these control records were taken. A similar experiment to that illustrated in fig. 16 was performed in only two other cones, and yielded essentially similar results.

To summarize, SRCI in cones as well as bipolar cells is blocked by KyA. To the extent that this effect reflects the abolishment of horizontal cell inhibition, this result strongly supports the model of fig. 3.

Part II.

Role of Horizontal Cell Coupling in SRCI.

The importance of horizontal cells in suppressive rod-cone interaction was clearly demonstrated in part I.

The horizontal cells of the retina receive input from photoreceptors via chemical synapses and also input from other horizontal cells via both chemical (Sakai & Naka, 1986) and electrical synapses (gap junctions) (Kaneko, 1971). As spelled out by a large number of previous investigators (e.g., Naka and Rushton, 1966; Lamb, 1976), the increase in the amplitude of horizontal cell responses to larger area of illumination is due to a lateral spread of current via these gap junctions, which thereby increases the receptive field of HC's beyond the area of receptors that provide direct input into the cell. SRCI recorded from horizontal cells, however, decreases when the size of the flickering test stimulus is increased, although the amplitude of flicker increases (Frumkes & Eysteinsson, 1987; Eysteinsson, Frumkes & Denny, 1987). It is therefore possible that the spatial limitations of SRCI may be dependent on the extent of electrical coupling of horizontal cells.

1) Effect of Dopamine on HC Coupling.

Since many previous investigations have suggested that dopamine uncouples horizontal cells in other species (Negishi & Drujan, 1978; Piccolino, Neyton & Gerschenfeld, 1984) and since there is some evidence for dopamine in mudpuppy retina (Adolph, Dowling & Ehinger,1980), dopamine would apparently be an appropriate agent for studying the role of HC coupling in

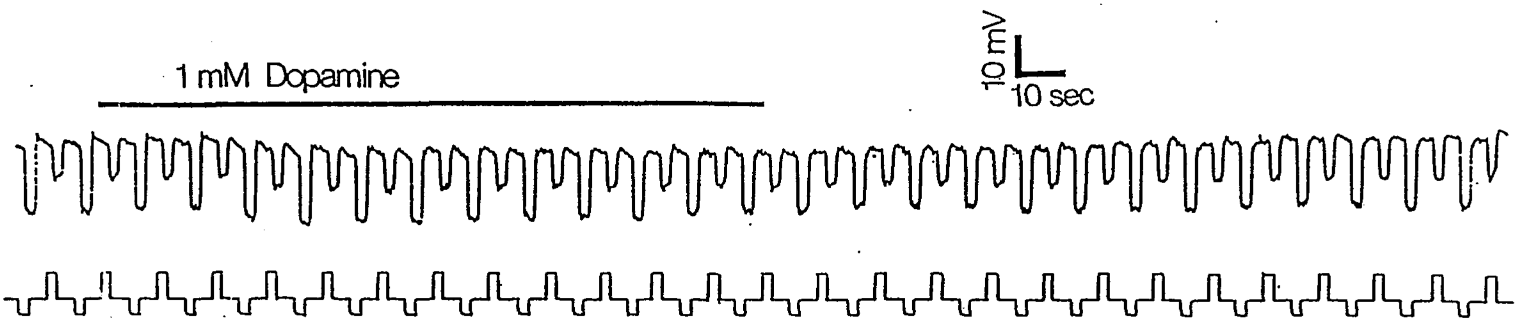
SRCI. Before investigation SRCI, however, it was necessary to first show in mudpuppy that HC coupling is influenced by dopamine.

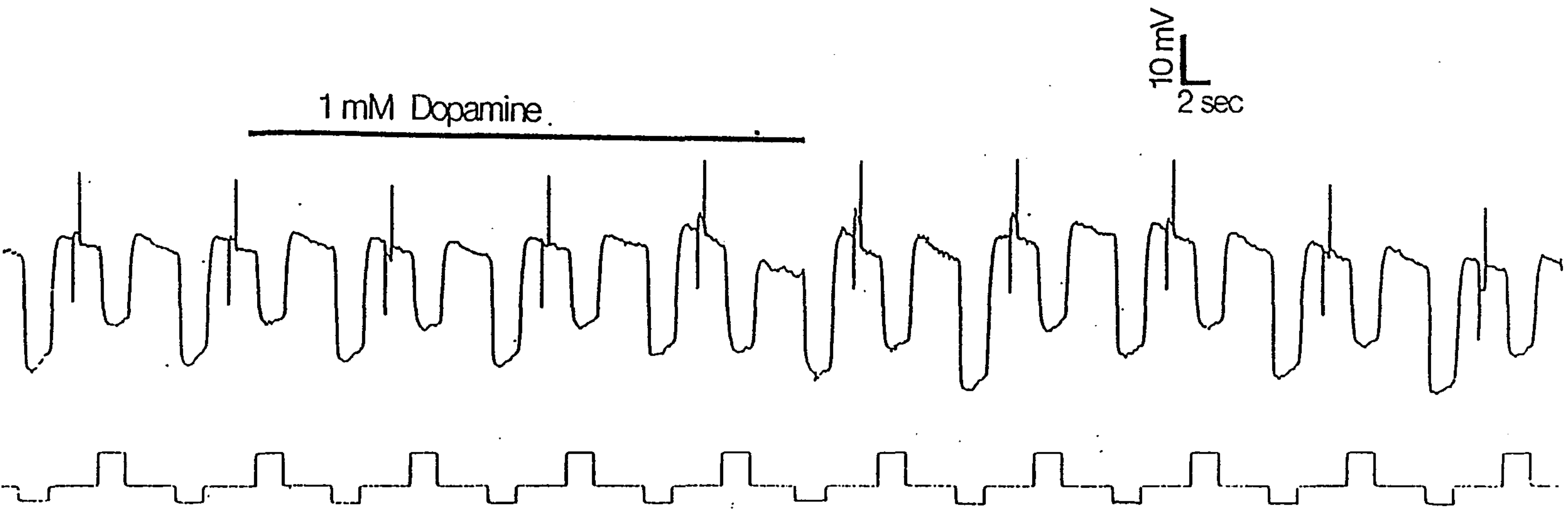
Figure 17 illustrates recordings from two mudpuppy horizontal cells. These responses were either elicited by the red LED or were 640 nm in wavelength as determined by an interference filter and are indicated respectively by an upward-going and downward-going stimulus trace. These stimuli were previously equated for visual effectiveness using diffuse light flashes to provide responses indistinguishable in waveform. However, for the illustrated data, the LED channel was used to provide a 430 um spot centered around the recording electrode.

The records on the left in fig. 17 show that in control media, the diffuse light elicits a much larger response than the spot of light. After obtaining several control responses, 1 mM dopamine was introduced as indicated by the long horizontal bar over the responses. For the cell illustrated on top notice that dopamine causes a slight increase in the response evoked by the small spot of light and a decrease in the response to the diffuse light, as well as a slight hyperpolarization of the dark membrane potential. These effects reversed within 2 minutes upon return to control Ringers.

Dopamine similarly induced a decrease in the ratio

Figure 17. Intracellular recordings from two mudpuppy horizontal cells. The responses were to two stimuli, a 230um circular spot of light, centered with respect to the electrode, and a diffuse stimulus. Both were 640nm and equated for intensity. Presentations of the small spots are indicated by upward deflections on the stimulus trace. For both cells, 1 mM Dopamine was superfused during the time period indicated by the horizontal bar above the records.


1 mM Dopamine
10 mV
10 sec


1 mM Dopamine
10 mV
2 sec

of the amplitude of the responses evoked by the diffuse and focal stimulus in 20 of 22 horizontal cells investigated using either 1 mM or 500 um dopamine. However, there was inconsistency in other aspects of dopamine's effects. The influence of dopamine upon dark membrane potential was quite variable. The time course of dopamine's effects also proved quite variable. For most cells, dopamine's influence took about 3-4 minutes to fully develop. In a few cells, however, dopamine's effect proved much faster as is the case for the bottom record in figure 17. In addition, the influence of dopamine upon the relative magnitude of responses to focal and diffuse stimuli was quite variable. Thus, it could be small or pronounced as indicated, respectively, by the upper and lower records in figure 17.

In the case of 9 horizontal cells, +0.1 nAmp current pulses were presented through a bridge device to assess the influence of dopamine upon the input impedance of the HC, as is the case for the cell illustrated on the bottom of figure 17. By Ohm's law, it follows that a 1 mV change in the response to this current pulse represents a 10 megohm change in input impedance. Notice that for the illustrated cell, dopamine cuases an approximate 50 megohm increase in input impedance. A value very close to this was obtained in the other 8 cells so investigated. Although I did not measure the input

impedance of these cells, Nelson (1973) reported that mudpuppy horizontal cells tend to have an input impedance of 30 megohms. Thus, an increase of 50 megohms represents a considerable increase in input impedance, a result which could easily reflect a decrease in electrical coupling between horizontal cells. Therefore, in combination with the changes in the relative amplitude of responses to focal and diffuse light flashes, these data are consistent with the idea that dopamine probably uncouples HCs and is therefore, an appropriate agent for use in SRCI experiments.

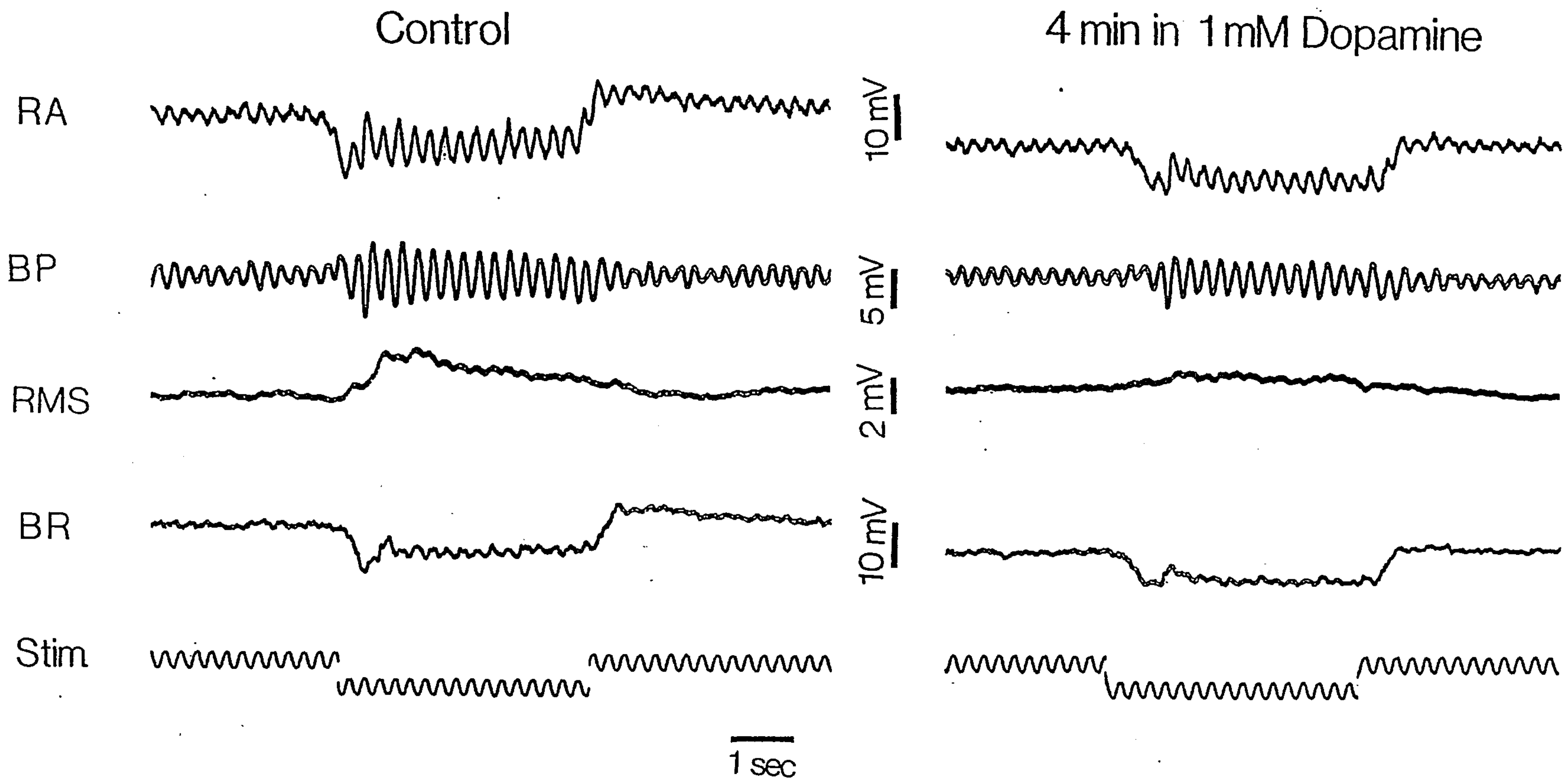
2) Effect of Dopamine on SRCI.

The influence of dopamine upon SRCI in a HC are illustrated in fig. 18, and the same paradigm used to study other drugs in figs. 9 through 15 was used in this experiment. The left panel of data clearly show background enhancement of flicker (seen most clearly in the BP and RMS records): also the BR record shows that photic stimulation of the receptive field area surrounding the one stimulated by the focal stimulus causes a hyperpolarization, just as when stimulation is only centered around the recording electrode. After obtaining these data, dopamine was introduced for four minutes at which time I obtained the data illustrated in the right panel. Dopamine causes no reliable influence

upon flicker responsiveness when no adapting field is present. With no adapting field present for the cell illustrated in fig. 18, it produces a 5mV hyperpolarization (seen in the RA and BR record) and a <10% overall decrease in the focal flicker response (see the BP record). Dopamine's most pronounced effect is to decrease the influence of the diffuse background upon flicker as seen most clearly in the RMS and BP records. In other words, dopamine considerably reduces SRCI.

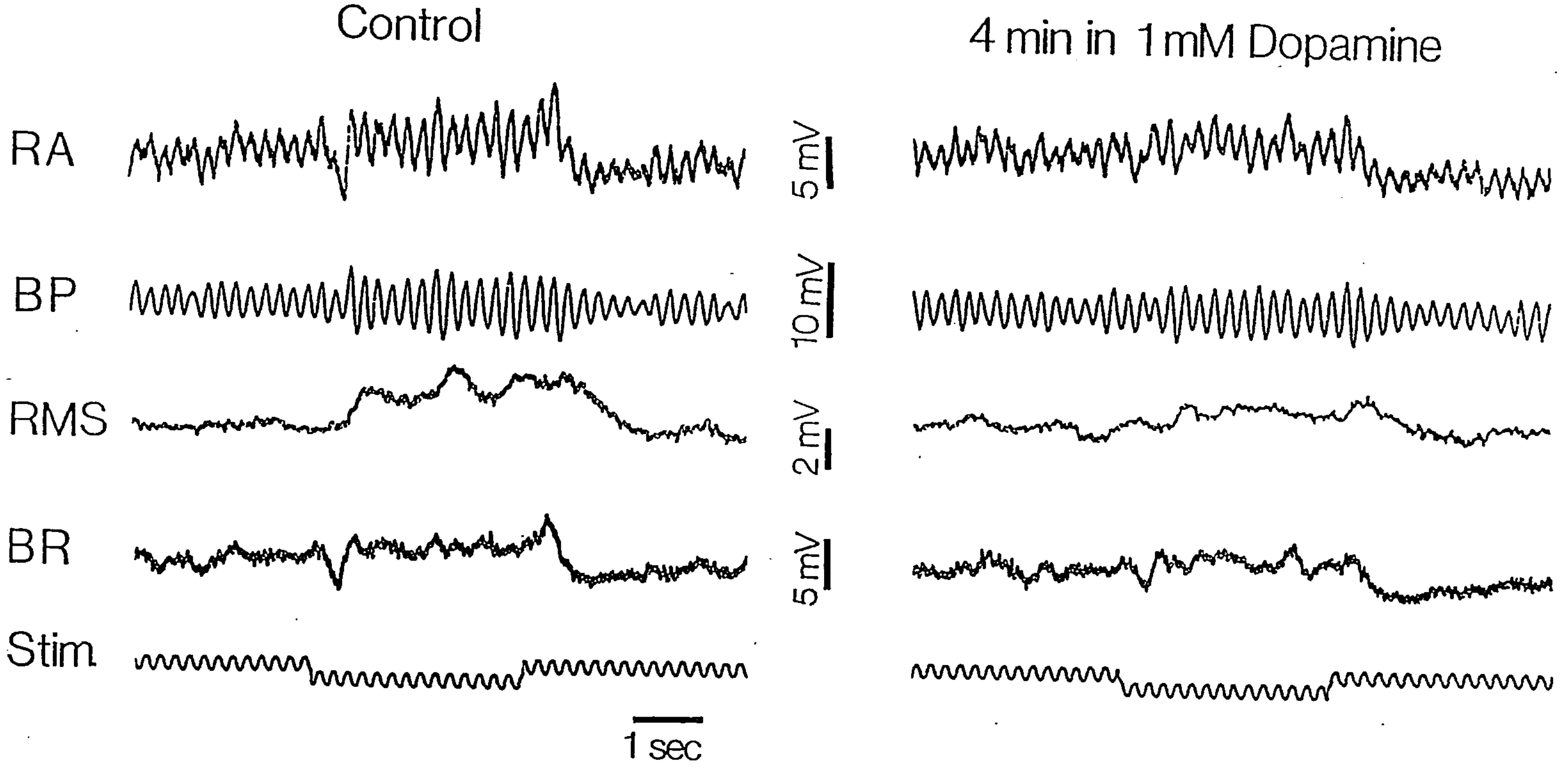
If dopamine reduces SRCI in any cell type, the model in figure 2 would predict that it should reduce it in all cell types. This proved to be the case in the only other neuronal type I investigated, a hyperpolarizing bipolar cell illustrated in figure 19. Other than the type of cell recorded, the illustrated experiment was virtually identical to that used with the HC in fig. 18 above. The left records obtained in control clearly show an antagonistic surround (seen most clearly in the BR records) as well as obvious background ehancement of flicker. Following 4 minutes of exposure, dopamine produced a very slight change in flicker amplitude in the dark (seen most clearly in the BP record). Of considerably greater interest is the reduced background enhancement of flicker (seen most clearly in the BP and RMS recoreds), and the reduced antagonism of the surround mechanism (seen most clearly in in the BR record). Since

Figure 18. Intracellular recordings from a mudpuppy horizontal cell. The left panel of recordings shows SRCI recorded from the cell in control Ringer's, using the stimulus paradigm as in figure 16, except the flicker frequency was 5 HZ. Note one temporal calibration bar for all recordings, while separate calibration for each row of recordings. All abbreviations are the same as in figure 16. The left panel shows a comparable recording from the cell after 4 minutes superfusion of dopamine.



Control
4 min in 1 mM Dopamine
RA
BP
RMS
BR
Stim
10 mV
5 mV
2 mV
10 mV
1 sec

Figure 19. Intracellular recordings from a mudpuppy hyperpolarizing bipolar cell (HPBC). Same stimulus paradigm and abbreviations as in figure 19, i.e a continuous red focal flickering stimulus was used, and 3 seconds presentation of a dim, 520nm background as indicated by a downward deflection on the stimulus monitor. The left panel was taken during superfusion of control Ringer's, while the right panel after 4 minutes superfusion of dopamine.


Control
4 min in 1 mM Dopamine
RA
BP
RMS
BR
Stim
5 mV
10 mV
2 mV
5 mV
1 sec

this was the only bipolar cell studied in the presence of dopamine, I can not comment on the reliability of these effects. However, the influence of dopamine upon SRCI is consistent with an uncoupling action upon HCs (see discussion).

In summary, dopamine reduces SRCI in horizontal and bipolar cells. Unlike the action of D-O-phosphoserine, Kynurenic Acid, or lead, this reduction in SRCI is <u>not</u> associated with a marked increase in flicker responsiveness with no background field present.

Part III.

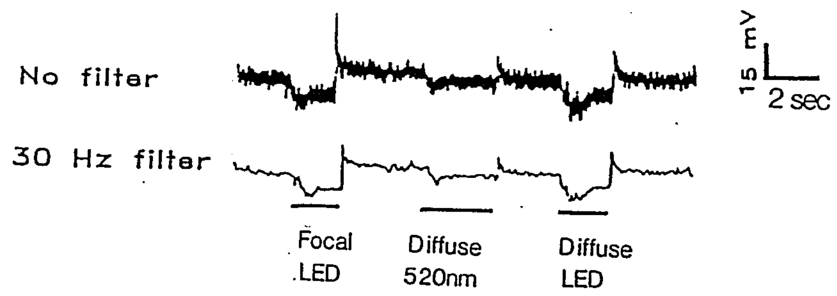
SRCI in the Inner Retina.

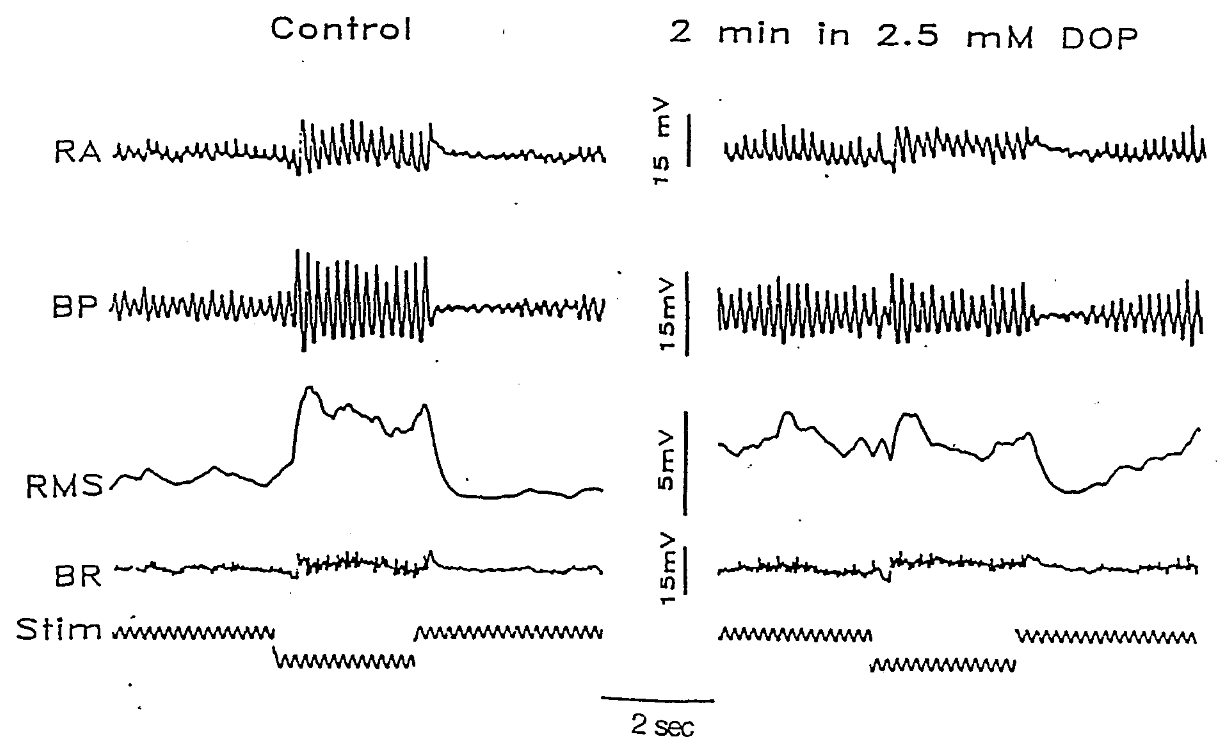
The model in figure 3 portrays the inner retina as an entity which relays information which is fully processed in the distal retina, and that any new response properties that arise in the inner retina are secondary to the distal processing. Accordingly, processing that has taken place in the outer retina will help determine the response properties of inner retinal neurons, including the presence or absence of SRCI. It follows then from this model that any of the treatments which block SRCI in the distal retina should have a similar effect in inner retinal neurons. It also follows

logically that treatments which fail to block SRCI in the distal retina should fail to block SRCI in the inner retina.

In the course of this dissertation, I did record from some cells which responded as predicted. For example, figure 20 shows recordings from an inner retinal neuron whose responses to focal and diffuse 2 second duration flashes is indicated in the upper left. Based upon criteria established by Coleman (1983) in HRP stained neurons, this spiking OFF-neuron is most probably an amacrine cell. Because the response of this neuron is dominated by spikes, I "preprocessed" this data with a low pass filter with a 30 hz cutoff to obtain a response more similar to that of distal retinal neurons, the second row of tracings from the top. The data on the bottom of the figure is from a usual SRCI experiment and is equivalent to that for bipolar cells and cones. The left panel shows the presence of SRCI and an antagonistic surround. In fact, these data are similar to that from the hyperpolarizing bipolar cell (e.g., fig. 12) which is likely to be its presynaptic excitatory input (see Frumkes, Miller, Slaughter & Dacheux, 1981). The BP record clearly shows background enhancement of flicker and the BR record, an antagonistic surround. After obtaining these data, I superfused the retina with 2.5 mM DOP. I chose this particular concentration since it is

Figure 20. Intracellular recordings from an OFF-amacrine cell in mudpuppy retina. The two uppermost traces show responses to a focal red LED, a diffuse 520nm stimulus, and a diffuse red LED. The lower trace shows the same responses after filtering with a 30Hz bandpass filter, which was also used for the two lower panels of records. The panel on the left shows recordings from the cell in response to a focal red flicker stimulus and one presentation of rod-adapting background. The panel on the right shows a similar recording after 2 minutes superfusion with 2.5mM D-O-Phosphoserine (DOP). Abbreviations the same as before. Note separate amplitude calibration markers for each amplifier output.


No filter
30 Hz filter
15 mV
2 sec
Focal LED
Diffuse 520nm
Diffuse LED


Control
2 min in 2.5 mM DOP
RA
BP
RMS
BR
Stim
15 mV
15mV
5mV
15mV
2 sec

known to influence horizontal cells (Slaughter and Miller, 1985) but is too low a dosage to have any direct effect in the inner retina (Slaughter and Miller, 1985). The right panel was obtained after 2 minutes superfusion in 2.5 mM DOP. As is the case for hyperpolarizing bipolar cells, DOP enhances flicker in the dark, reduces background enhancement of flicker, and decreases the antagonism attributable to the receptive field surround if this neuron. Experiments with this cell are entirely consistent with the simple model presented in fig. 3.

However, even before performing the present thesis, I did not believe that SRCI would remain unaltered by inner retinal neuronal circuitry. For example, Frumkes and Eysteinsson (1987) reported that most on-off amacrine and ganglion cells failed to demonstrate SRCI, suggesting that some inner retinal processing alters SRCI.

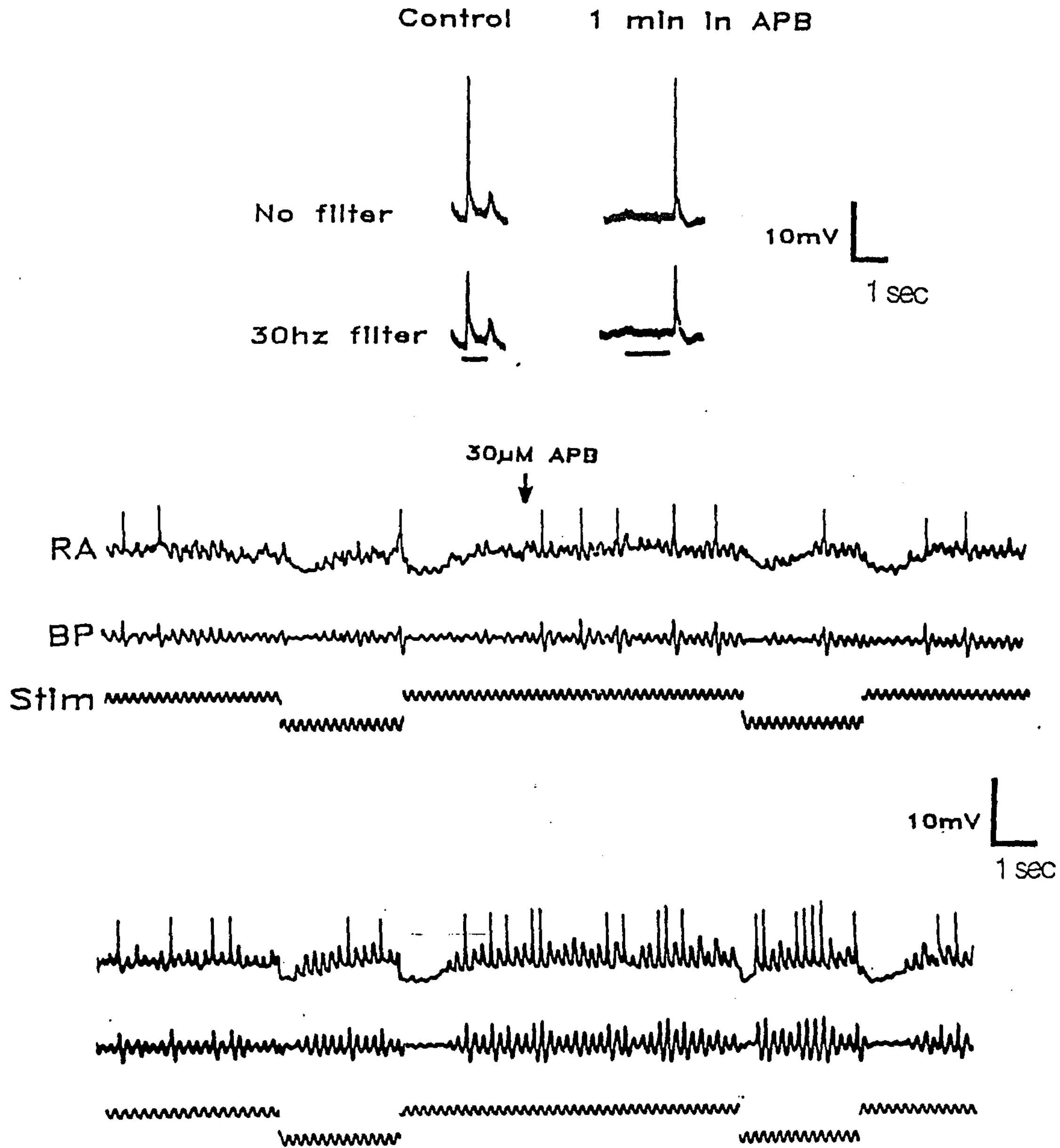
In this thesis, I decided to test the model in fig. 3 by applying pharmacological agents which had no effect upon SRCI in distal retina while recording from proximal neurons. If the model is correct, such substances should have no effect upon SRCI recorded in proximal neurons. I decided to use 2-amino-4-phosphonobutyric acid (APB), a substance which is known in mudpuppy to block the response of depolarizing bipolar cells but no other class of distal retinal neuron (Slaughter and Miller, 1981).

In the appendix, I show that although APB slightly enhanced flicker, it has no reliable influence on SRCI in horizontal cells. Since the model in fig. 3 indicates no specific role for depolarizing bipolar cells in determining the responsiveness of cones, horizontal cells, and hyperpolarizing bipolar cells, it follows that APB should have no specific influence in off- and on-off inner retinal neurons. Obviously, APB should block all responses in cells receiving their principal excitatory input from the depolarizing bipolar cells, on-amacrine and on-ganglion cells.

Figure 21 shows data from an experiment on a mudpuppy ON-OFF neuron, most probably a ganglion cell (see Coleman, 1983). The top two traces on the left show the response of this neuron to a 2-second duration diffuse light flash. Note the response waveform is dominated by an ON-EPSP and spike, but an OFF-EPSP is also evident. After 1 minute presentation of APB, the on response is almost totally lost, and the response consists of an off-EPSP associated with a large spike. This treatment totally reversed three minutes after return to control media.

I then began to study SRCI in this cell, using the same stimulus parameters used while recording from bipolar cells. This experiment is indicated in the

Figure 21. Intracellular records from an ON-OFF ganglion cell in mudpuppy retina. The two upper traces show recordings in response to 2 seconds light pulses. The left records are taken in control Ringer's, while the records on the right are taken after 1 minute superfusion with 30um APB. Note separate calibration markers for these on the right. The lower traces show the same responses as the upper ones, but after feeding them through a 30Hz bandpass filter, which was also used for the continuous records below. The two lower rows of records are responses to a continuous focal flicker stimulus and four consecutive presentations of a dim 520nm background. At the time indicated by arrow, the superfusing medium was switched to 30um 2-amino-4-phosphonobutyric acid (APB). Abbreviations same as before.


Control
1 min in APB
No filter
30hz filter
10mV
1 sec
30µM APB
RA
BP
Stim
10mV
1 sec

bottom two sets of traces. In this figure, these lower data were preprocessed with the 30 hz cutoff filter. The three traces represent the ouput of the 30 hz filter (labeled "RA), the BP output, and the stimulus trace. Notice in the upper left that flicker responsiveness is unaltered by presentation of the diffuse backgound, which does, however elicit some ON- and OFF-hyperpolarizations most probably reflecting IPSPs. APB enhances flicker in the dark, and more interestingly, the cell now shows a background enhancement of flicker responsiveness, SRCI. This is evidenced both by considering the amplitude of slow potentials (the BP record) or by considering the prevalence of spikes (the RA record). The simplest possible interpretation for this observation is that SRCI is not evident in control media in ON-OFF cells, merely because ON-and OFF-excitatory responses are constantly cancelling each other other out. Since flicker responsiveness is never evident, SRCI is not evident. This arguement is consistent with similar results obtained from several other ON-OFF amacrine and ganglion cells. Thus, although it is possible that inner retinal circuitry more profoundly alters SRCI, I have obtained no evidence for this.

Chapter IV: General Discussion.

The main results from the present study can be sumarized as follows.

1. SRCI in all neuronal types can be blocked by agents which block the photic response of horizontal cells (DOP, KyA, PDA). These agents enhance flicker to levels normally only observed in the presence of selective rod-light adaptation.

2. Mudpuppy cones have a receptive field surround which is attributable to rod input. This surround mechanism, as well as SRCI in cones, is blocked by the same pharmacological agent (KyA) that blocks SRCI in second order neurons. Therefore at least in part, SRCI reflects rod-modulation of horizontal cell feedback onto cones.

3. Lead chloride selectively blocks the rod-input to horizontal cells while leaving the cone input intact. Lead blocks SRCI observed in horizontal cells by enhancing flicker response to levels normally only observed in the presence of selective rod-light adaptation.

4. Dopamine as well as its precursor, L-DOPA, partially uncouples horizontal cells in mudpuppy. Dopamine has a

very small effect upon flicker responses in the dark but reduces the enhancement of flicker produced by selective rod-light adaptation.

5. SRCI in third order neurons seems to reflect processing in the distal retina. Thus, in ON- and ON-OFF cells, it is blocked by agents which blocks it in bipolar cells (DOP or KyA). Although on-off neurons usually fail to display SRCI, this probably reflects destructive interference between the ON- and OFF-responses within this neuron. Thus, SRCI can be observed in ON-OFF cells when the ON-input is blocked with APB.

In the following discussion, I consider each of these points separately. At the conclusion of this discussion section, I then discusss the relationship of SRCI to receptive field surround mechanism.

1. Horizontal Cells Mediate SRCI

The present results clearly show that SRCI is blocked in all neuronal types by pharmacological agents such as KyA or DOP which block horizontal cell responses. Furthermore, SRCI in horizontal cells is similarly blocked by suppressings rod-input to horizontal cells. Finally, agents such as APB (fig. 21) or picrotoxin or

bicuculline (unillustrated observations) with no known influence upon horizontal cells fail to have any specific influence upon SRCI. The simplest and most logical conclusion is that SRCI is mediated by horizontal cells.

Assuming that horizontal cells mediate SRCI, it is far from clear whether they do so by means of a feedback (onto cones) or feedforward pathway (onto bipolars) or both. Since SRCI in cones is blocked by KyA, it is clear that SRCI in cones reflects a feedback pathway. There remains the possibility that SRCI additionally reflects a feedforward influence. Unfortunately, with the present pharmacological approach and with the pharmacological agents used, it is impossible to determine whether SRCI additionally reflects a feedforward influence. The possibility of exploring the relatived importance of feedback vs. feedforward pathways is discussed further below.

The present experiments also leave unexplained the temporal limitations to SRCI (e.g., the inability to observe it with long duration pulses or slow frequency flicker) or the inability to observe it in color-coding second order neurons (see Frumkes and Eysteinsson, 1987). Perhaps these results would be better studied by means of an approach involving simultaneous recording and

stimulation from nearby pairs of neurons (e.g., Attwell, 1986) and/or by using pharmacological agents which interfere with electrically excitable membrane properties.

Finally, the observation that SRCI is interfered with by dopamine suggests that in addition to a distal retinal circuit, SRCI is further modified by an interplexiform circuit. In fact, there is some suggestive evidence for a dopaminergic interplexiform cell in mudpuppy (Adolph, Dowling, & Ehinger, 1980). The importance of dopamine is discussed further below.

2. Feedback onto Mudpuppy Cones.

One of the more striking results of the present study (as well as those of Frumkes and Eysteinsson, 1987) is the clear evidence of a surround mechanism in mudpuppy cones. Perhaps the failure of previous investigations (e.g., Werblin and Dowling, 1969; Fain, 1975) revolves around the rod-domination of this surround mechanism. Thus, since this study attempted to explore rod-cone interaction, I was predisposed to look for a result which might have appeared unlikely to prior investigators.

It is unlikely that this surround mechanism can be attributed to anything other than horizontal cell input,

since KyA and PDA have been reported to have no direct effects upon photorecpetors (Slaughter and Miller, 1983; Miller, Slaughter, Coleman & Massey, 1987). Thus, these results indicate quite clearly the mudpuppy cones, like cones in fish (Yazulla, 1983) and turtle (Baylor, Fuortes, & O'Bryan, 1971), receive feedback.

Some previous pharmacological studies explored the possibility that SRCI involved GABA or glycine action. In *Xenopus*, these transmitters have been shown to modify the balance of rod and cone inputs into horizontal cells in opposite fashion (Stone & Witkovsky, 1984; Witkovsky & Stone, 1987). Furthermore, there is some evidence in *Xenopus* (Witkovksy and Stone, 1987) and tiger salamander (Wu, 1986) that GABA mediates horizontal cell feedback onto cones. *If* we assume that feedback is GABAergic in mudpuppy as well as in *Xenopus* and tiger salamander, this suggests that SRCI recorded in cones should be blocked by GABA antagonists such as picrotoxin or bicuculline. If SRCI was observed in second order neurons in the presence of GABA antagonists, this would suggest that two types of horizontal cells mediate SRCI: GABAergic cells feeding back to cones and non-GABAergic cells feeding forward onto bipolars. Without futher data, it is difficult to speculate any further. Indeed, some available data clearly argues against this possibility. Specifically,

Miller, Frumkes, Slaughter & Dacheux (1981) reported that mudpuppy cones are not influenced by GABA or its antagonists. Additionally, application of GABA antagonists failed to influence SRCI recorded in Xenopus (Eysteinsson and Frumkes, 1985) or cat (Pflug and Nelson, 1986) horizontal cells.

3. Lead Chloride Blocks Rod-Input to Mudpuppy Horizontal Cells

Rod input into horizontal cells is determined by the level of rod dark adaptation and the rod receptor potential. The effect of lead chloride on rod activity as measured by ERGs (Fox and Sillman, 1979) can be explained by its effect on the rod receptor potential (Tessier-Lavigne, Mobbs, & Attwell, 1985). The present study also shows that lead blocks the rod-mediated photic response in horizontal cells. As would be expected, this loss of rod-input selectively blocks SRCI. In fact, the present SRCI study suggests one mechanism for lead's effects. Since flicker responses in the dark are enhanced by lead, this suggests that lead acts by blocking the rod-released neurotransmitter which normally activates inhibition upon cone-pathways. Although not previously claimed for lead ions, Evans, Hood & Holtzman (1978) previously suggested that low concentrations of cobalt or cadmium ions have such an effect in the retina

of another amphibian, Rana. Although this possibility should be considered, it must be stressed that if this were lead's only effect, horizontal cells should always hyperpolarize in the presence of lead. In fact, I observed that lead had a variable influence upon the dark membrane potential of horizontal cells. Thus, it is likely that lead has a number of unknown influences upon rods in addition to those found by Tessier-Lavigne, Mobbs and Attwell (1985), and perhaps on other cell types that I did not examine.

4. Horizontal Cell Coupling and SRCI.

If horizontal cells mediate SRCI, the spatial limitations to SRCI should reflect in some way, the receptive field properties of horizontal cells. Indirectly, this possiblity is indicated by observations made in cat (Pflug and Nelson, 1986) and amphibian (Frumkes and Eysteinsson, 1987) retina. SRCI is best observed with small flickering test stimuli: as the size of the flickering test probe increases, the relative amplitude of the flicker response obtained in the presence of the background (in respect to that obtained with no background) decreases. This result can be explained as follows. In the typical SRCI experiment, the flickering test stimulus is more than 2 log units (i.e., a factor of 100) brighter for rods as well as

cones than the rod background stimulus. Therefore, the rod adapting background does not appreciably alter the illumination of the retinal area immediately under the test stimulus, only the surround retinal area. Consequently, as the test stimulus size increases, the smaller the effective area of the retina that is influenced by the background. If one assumes that the effective influence of the background monotonically increases with its effective area, it logically follows that background enhancement should increase as flicker test probe size increases.
Using this line of reasoning, Eysteinsson, Frumkes, and Denny (1987) have related the spatial limitations of SRCI to the RC coupling properties of the electrically coupled horizontal cell network.

This type of explanation can also be attacked pharmacologically. In the present study, I show that dopamine partially uncouples horizontal cells. This uncoupling amounts to decreasing the effective area of a background which will influence a horizontal cell's response to light. Thus, dopamine should decrease the background's effect upon flicker, an observation which is in agreement with data obtained in the present study from horizontal and bipolar cells. Of interest, unlike lead or amino acids which clearly enhance flicker responses in the dark, dopamine has but a slight effect upon flicker

responsiveness in the dark. Thus, regardless of whether the above explanation is correct, this suggests that dopamine blocks SRCI in a different manner than amino acid antagonists or lead.

As pointed out earlier, dopamine has been found localized in neurons in the inner plexiform layer of the mudpuppy retina (Adolph, Dowling & Ehinger, 1980). In many other species, a dopaminergic interplexiform cell has been located which presumably plays an important role in light and dark adaptation (Mangel and Dowling, 1987). Thus, dopamine has been found to reduce the receptive field of horizontal cells teleost fish (Negishi & Drujan, 1978) and to block the diffusion of fluorescent dyes such as Lucifer yellow between horizontal cells via gap junctions in turtle (Piccolino, Neyton & Gerschenfeld, 1984). Similar effects result from prolonged dark adaptation, suggesting that dopamine plays a key role in dark adaptation (Mangel & Dowling, 1985). Some similar effects have recently been noted in _Xenopus_ retina (Witkovsky, Stone & Besharse, 1987).

These observations suggest that the present effects of dopamine reflect a role for a dopaminergic interplexiform cell in SRCI in mudpuppy. Indeed, the fact that L-DOPA produces a similar effect upon SRCI as dopamine (see appendix) might indicate that some of the

metabolic machinery necessary for dopamine synthesis is present in the retina. Three factors tend to rule out a very strong role of dopamine. First, the number of dopaminergic units in mudpuppy is much less than in other species (Adolph, Dowling & Ehinger, 1980). Secondly, the concentrations of dopamine used in this study are much higher (a factor of 100) than those used in other species. Thirdly, SRCI in mudpuppy retina is quite rapid: unless extensive photopigment bleaching occurs, background enhancement of flicker is complete within a few hundred msec. of stimulus onset, and its effects reverse within a few seconds. In contrast, dopamine effects in fish, turtle, and <u>Xenopus</u> take at least one half hour and as long as two hours. Thus, although it is possible that dopamine and/or an interplexiform cell plays a role in SRCI, possibly by modulating it, the present results neither support or refute such a possibility.

5. Possible Role for Inner Retinal Proceessing in SRCI.

The model presented in figure 3 assumes that the inner retina plays no role in SRCI and merely relays processing completed within the outer retina. Previous recordings of SRCI in both *Xenopus* and mudpuppy inner retinal neurons (Frumkes & Eysteinsson, 1987) showed that SRCI in ON- and OFF-ganglion cells seem to reflect SRCI

in the cells which consitute their presumed excitatory input, respectively the depolarizing and hyperpolarizing bipolar cell. Thus for example, SRCI in depolarizing bipolar cells is usually less than in hyperpolarizing bipolar cells: among third order neurons, SRCI is less obvious in ON- than in OFF-ganglion cells. Along these same lines, the present results show that pharmacological agents which block SRCI in bipolar cells produce similar effects in on- and in OFF-ganglion cells cells.

However, Frumkes and Eysteinsson (1987) found few ON-OFF amacrine and ganglion cells which would demonstrate SRCI suggesting some role for inner retinal neural processing. A priori, one might expect two types of processing. First, the failure to obtain SRCI under control circumstances might merely reflect constructive inteference between ON- and OFF-excitatory input in ON-OFF cells. Thus, in response to a flickering stimulus, the addition of ON-excitation is counteracted by a removal of OFF-excitation and vice-versa. As a consequence, ON-OFF cells respond badly under any circumstances to flicker, and therefore do not show SRCI. According to this explanation, one would anticipate that pharmacological removal of ON-excitation with APB (Slaughter and Miller, 1981) should alter the reponse of an ON-OFF cell so that it resembles that of an OFF-cell, and should demonstrate SRCI. In fact, this proved to be

the case in all ON-OFF cells I examined with APB (e.g., see fig. 22). However, Belgum, Dvorak, McReynolds, and Miyachi (1987) proposed that the response of mudpuppy ganglion cells reflects a complex interplay between excitation and disinhibition and inhibition and disfacilitation. Depending upon the relevative synaptic strenghth of inhibition and excitatory synapses, one might therefore anticipate any one of a number of other possible effects of APB upon SRCI in ON-OFF cells. Although all cells recorded could be interpreted in terms of a destructive interference between ON- and OFF-excitation, my sample size was small. Therefore, possible additional roles of inner retinal neurons in SRCI cannot be excluded.

Relationship of SRCI to Receptive Field Surround Mechanism

In the present thesis, amino acid agents which attenuated or abolished SRCI reduced the antagonistic receptive field surround of the recorded neuron. This might suggest that SRCI is merely a reflection of the receptive field surround mechanism and is of no further interest. This line of reasoning, however, fails on one ground. That is, only rod-adaptation produces this influence upon cone-flicker, an influence which cannot be duplicated by cone-adaptation. Although this rod-

specificity cannot be explained, it is highly reliable. Since in mudpuppy bipolar cell surround mechanisms have both rod- and cone-input (Fain, 1975), SRCI must in part reflect some process which is indepedent of the usual measure of the surround.

Over the years, there have been a number of reports of enhancement effects attributable to adaptation. For example, Burkhardt (1974) reported in mudpuppy that small diameter background fields had a greater adapting influence upon a particular neuron's response than stimuli that adapted the whole retina. This effect had no direct relationship to the recorded neuron's receptive field as it was as obvious in cells with and without antagonistic receptive field surrounds (bipolar and horizontal cells respectively). More recently, Itzhaki and Pearlman (1987) have attributed similar results to direct electrical coupling between cones and rule out the possibility of any post-receptor involvement. It should be stressed that Burkhardt's data were obtained with long duration stimuli under photopic adaptation condition. Thus, the relationship of these data to SRCI is obscure.

Using another approach, Chappell, Naka, Ripps, and Saukuranaga (1986) in skate and Chappell, Naka, and Sakuranaga (1985) in turtle have shown a background enhancment of flicker within similar temporal limitations

to those we observe for SRCI. In turtle, they report this phenomenon only involves cones and is _not_ dependent upon HC feedback. Since the skate retina presumably contains just rods and feedback onto rods is unknown, this observation must also reflect a phenomenon somewhat distinct from SRCI.

The data just summarized indicate the existence of phenomena which in some respect, bear some similarity to SRCI. The non-linear analysis used by Chappell and co-workers has not yet yielded an explanation for background enhancement of flicker. But although the pharmacological approach currently used might at first seem more successful, it has also failed to answer the most obvious question of all: why rod backgrounds, not cones, influence cone-flicker. Different methods and techniques are needed to address that question and other related ones in addition to or in combination with the pharmacological approach. This study has merely been a single chapter in that continuous research effort.

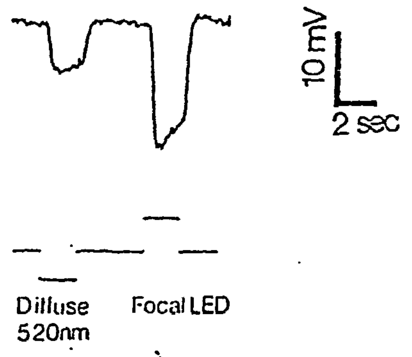
Appendix I.

During the course of the experiments reported in the Results section above, I also collected a great deal of pharmacological data with no direct relevence to the model presented in fig. 3. A few of these experiments are reported here since they serve as controls (e.g., a specific drug action is required to produce a specific effect). In addition, they replicate the results reported by Slaughter, Miller and coworkers summarized in table 1 which are crucial for the interpretations offered in the results and discussion and section above.

Influence of APB upon Horizontal and Bipolar Cells

The results reported in Part III of the results section assume that the only influence of APB upon the distal retina is to abolish the response of DPBCs. Since APB has recentlly been reported to have some similar direct influence upon horizontal cells (Slaughter, 1986), I deemed it appropriate to study the influence of APB upon SRCI in these neurons. Typical results are illustrated in fig. 22, using a focal red flicker stimulus and a diffuse rod adapting background. 30um APB was superfused during the period indicated by the

Figure 22. Intracellular recordings from a mudpuppy horizontal cell. The top trace shows responses to a diffuse 520nm stimulus and a focal (230um) LED stimulus. Note separate calibration markers on the right for these responses. The lower recording shows a continuous record played at a slow polygraph sweep speed, during which a focal red flickering stimulus was continuously presented. A 520nm rod-adapting background was presented at 12 seconds intervals. 30uM 2-amino-4-phosphonobutyric acid (APB) was superfused during the time indicated by the long horizontal bar above the responses. Note the long (>1 minute) application of APB.


10 mV
2 sec
Diffuse
520nm
Focal LED

30µm APB
5 mV
10 sec

horizontal bar above the responses. The cell hyperpolarized and there is a general increase in both the response to the diffuse stimulus and to flicker. However, there seems to be no obvious influence upon background enhancement of flicker. Results in 5 other neurons were quite similar. In all cases, APB produced a slight hyperpolarization and some enhancement of flicker responsiveness. Without proceeding to a variety of other experiments, it is impossible to determine the mechanism(s) by which APB produced these effects: these could involve a direct influence upon either rods or cones, a directly influence upon HCs, or some type of synaptic input from DPBCs to HCs. Since APB has no specific influence upon SRCI, however, it is highly unlikely that results in inner retinal neurons are more likely to reflect the loss of an ON-input rather than less well documented influences of this drug.

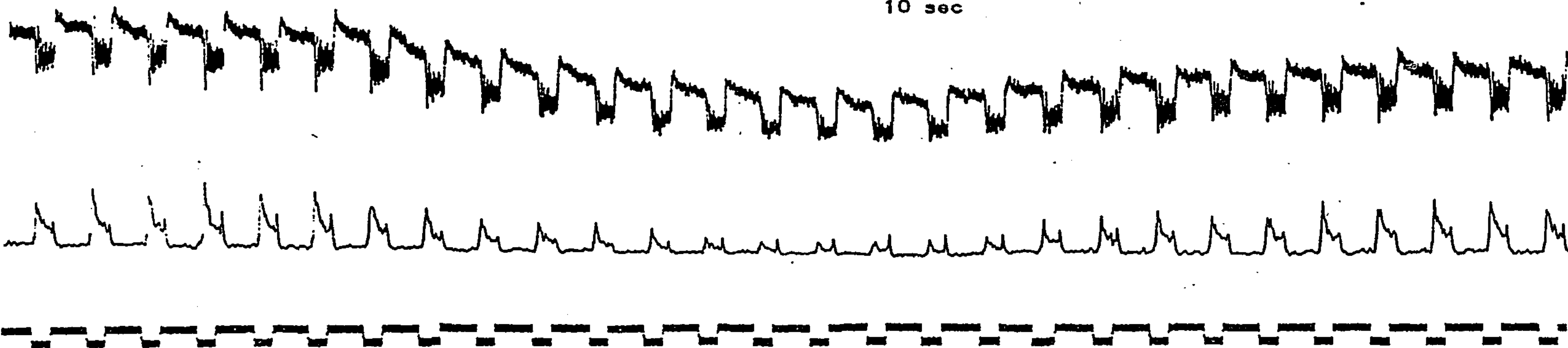
Although not illustrated, I also examined the influence of APB upon SRCI in bipolar cells. As reported by Slaughter and Miller (1981), APB blocked <u>all</u> photic responses in DPBCs, and had no obvious influence on any specific response component in HPBCs.

The influence of KyA and DOP upon SRCI in horizontal and Hyperpolarizing Bipolar Cells

Both D-O-Phosposerine and Kynurenic acid hyperpolarize horizontal cells and reduce the amplitude of the light response (Slaughter & Miller, 1983; Miller, Slaughter, Coleman & Massey, 1986). During the course of this study, I found that their effect on SRCI in horizontal cells is slightly different.

Figure 23 shows a continuous recording from a mudpuppy horizontal cell. The three rows of traces represent (from top to bottom) the output of the recording amplifier, the bandpass amplifier, and the stimulus trace. A focal (230um) red flickering stimulus was presented continuously, while a 520nm background stimulus was presented every 12 seconds, as indicated by the downward shifts in the stimulus trace. The initial records in control Ringer's solution shows a clear enhancement of flicker during background presentation. Then 5mM D-O-Phosphoserine was superfused during the period indicated by the long horizontal bar above the response record. The neuron hyperpolarized by about 7 mV and SRCI was sharply reduced, as seen best from the reduction of the RMS output, but the flicker response and background enhancement of flicker was never fully eliminated. Similar results were obtained from 5 other

Figure 23. Intracellular recordings from a mudpuppy horizontal cell. Note the slow sweep speed and low gain. A red sinusoidally flickering stimulus (5Hz) was continuously presented, and a 520nm background at regular intervals. The vertical bar over the upper record shows superfusion of 5mM D-O-Phosphoserine (DOP) on the cell. The middle trace shows the output of the RMS amplifier.

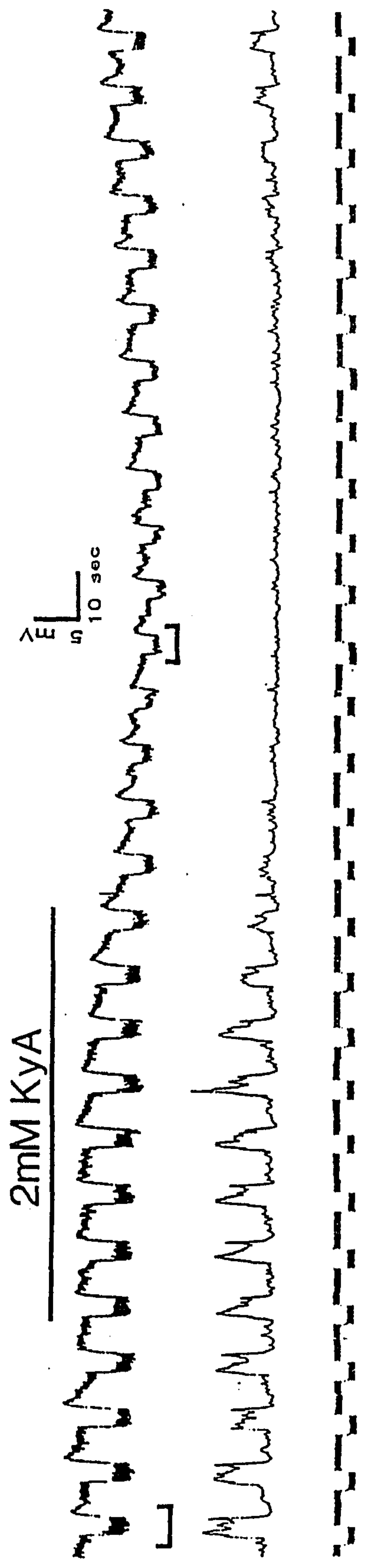

5mM DOP
5 mV
10 sec

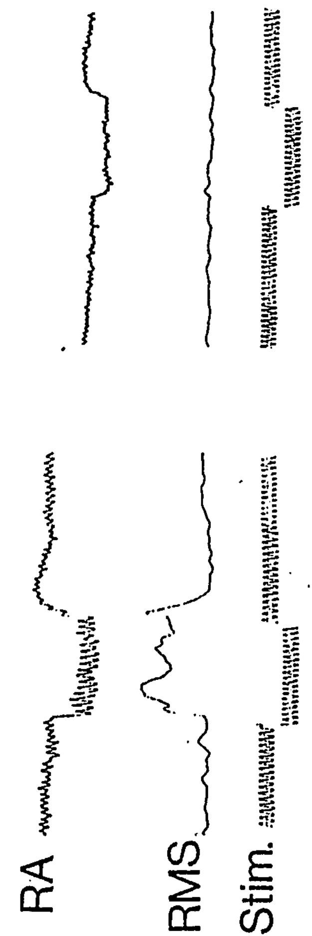
horizontal cells with DOP. A similar experiment using kynurenic acid was performed on several other mudpuppy horizontal cells, since KyA has similar effects on horizontal cell membranes. Figure 24 shows such an experiment. The upper part of the figure has the same format as figure 23. After 2 mM KyA was introduced, the cell hyperpolarized by about 7mV. Although a small response to the diffuse light is always apparent in the recording amplifier output, flicker responsiveness (as well as flicker enhancement) is totally abolished.

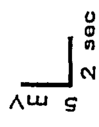
The two lower panels are the recordings taken at the time indicated by brackets in the continuous record, but played at a faster polygraph speed, in order to see more clearly the effect on the flicker response. The right panel, taken during the effect of KyA shows that the flicker response is completely eliminated by the treatment but not the response to the background. Kynurenic acid is clearly more potent than D-O-Phosphoserine on horizontal cells. But blocking SRCI in horizontal cells seems to be enough to block SRCI in second and third order neurons; eliminating the horizontal cell flicker response per se is not required. Thus, it is possible that some aspect of KyA's effect may additionally influence flicker responsiveness per se.

Kynurenic acid and Piperidine dicarboxylic acid (PDA) have similar effects on horizontal and hyperpolarizing bipolar cells (HPBC) (Slaughter & Miller, 1983a). It was

Figure 24. Intracellular recordings from a mudpuppy horizontal cell. The upper part of the figure shows a continuous recording from the cell. The top trace is the output from the recording amplifier (RA), while the middle trace is the output from the root-mean square (RMS) amplifier. A focal (230um) red flicker stimulus (5Hz) was continuously presented, while a 520nm background was presented every 12 seconds. 2mM Kynurenic acid was superfused during the period indicated by the horizontal bar. The two lower panels are records from the upper part indicated by brackets, but played at a faster polygraph sweep speed. Abbreviations as in previous figures. Note different calibration markers for each half of figure.


2mM KyA
5 mV
10 sec


Control
After 2mM KyA
RA
RMS
Stim.


5 mV
2 sec

therefore of interest to see if the effect of these on SRCI was different in HPBC's than horizontal cells. Figure 25 shows an experiment on a mudpuppy hyperpolarizing bipolar cell. The left panel shows the response to 6hz red flicker stimulus, 230um in diameter, and one 3 seconds presentation of a 520nm background stimulus. The bandreject (BR) amplifier output shows a clear antagonistic surround, while the RMS and BP output show a large SRCI and flicker response, respectively. While not shown, the cell hyperpolarized during PDA, and after 30 seconds, both SRCI and the flicker response were completely eliminated. But the response to background remains, although there was no evidence of the antagonistic surround during PDA action.

Dopamine, L-DOPA, and HC coupling

In the main text, I showed that dopamine partially uncoupled HCs. This suggests that dopamine may play some *in vivo* role in mudpuppy retina. Therefore, on five different horizontal cells, I examined the influence dopamine's precursor, L-DOPA, upon HC coupling. Fig. 26 is such an experiment using the same stimuli as shown in fig. 17, i.e. a diffuse and a focal red stimulus. The input impedance of the cell was monitored continuously by injecting a +0.1 nA current through the recording electrode between trains og light stimuli. After

Figure 25. Intracellular records from a mudpuppy hyperpolarizing bipolar cell. As in figure 12, the two top traces are responses form the cell to a diffuse 520nm stimulus and a focal red LED stimulus. Note the slow (2 sec) calibration marker for the sweep speed. The left panel shows a record from the cell, during superfusion of control Ringer's solution, in response to a focal red flicker and one presentation of the dim, diffuse 520nm background. The right panel shows similar records after a 30 seconds superfusion of the retina with 2mM piperidine dicarboxylic acid (PDA). The abbreviations on the left mean the same as in figure 6.

Diffuse
520 nm
Focal
LED

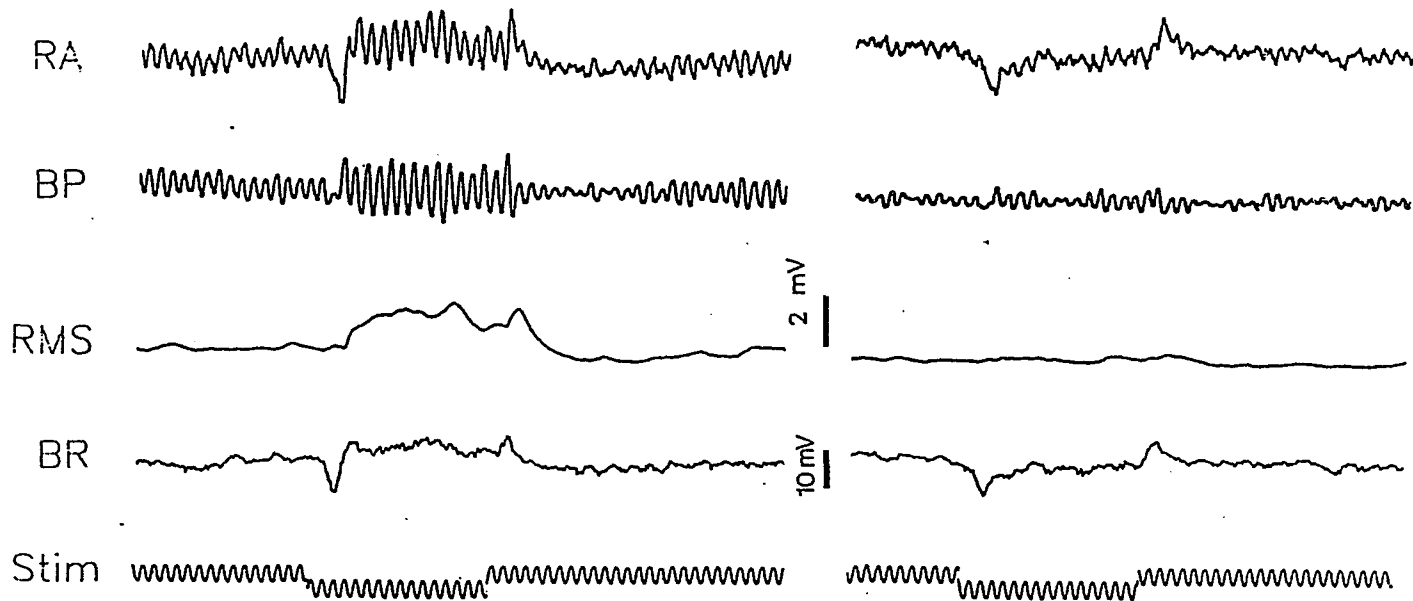
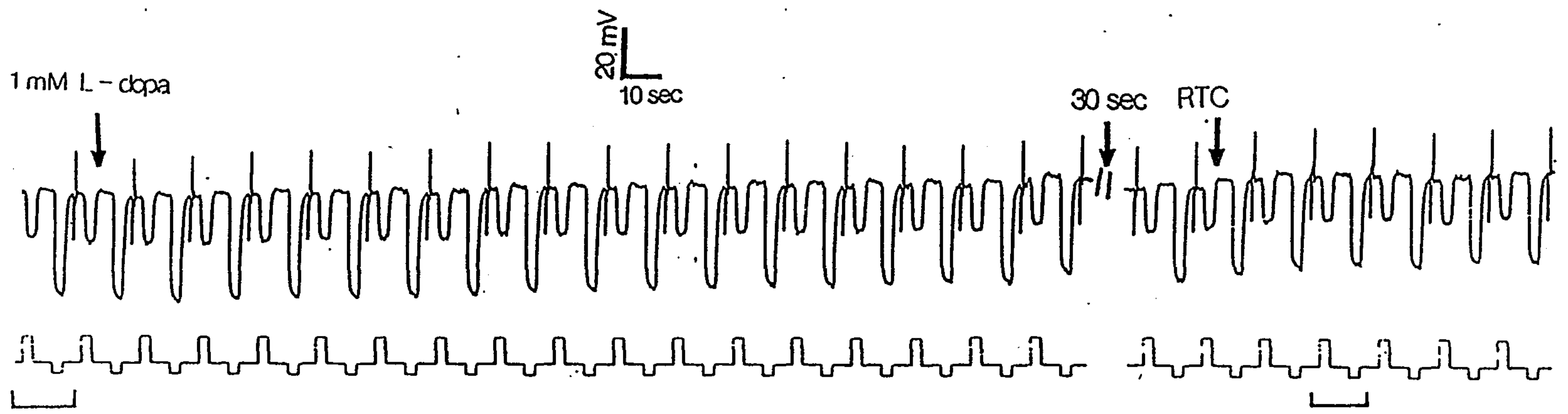
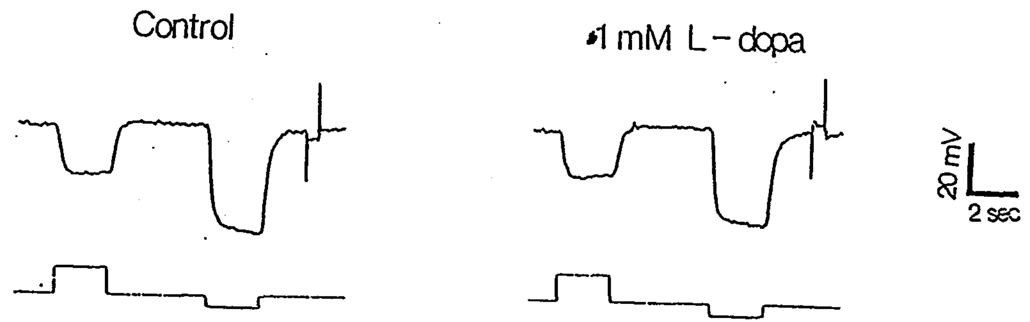

Control
30 sec in 2mM PDA
RA
BP
RMS
BR
Stim
2 mV
10 mV
10 mV
1 sec

Figure 26. Intracellular recordings from a mudpuppy horizontal cell. The same stimuli were used as in figure 17. A +0.1 nA current pulse was passed through a balanced bridge device into the cell between trains of light stimuli, to assess changes in input impedance. Superfusion of 1mM L-dopa was initiated at the point in time marked by an arrow on the left in the upper trace. Note the 30 seconds break in the record indicated by an arrow. The two lower panels are the recordings marked by the brackets in the slow record, but replayed at a faster polygraph speed.


1 mM L-dopa
20 mV
10 sec
30 sec
RTC


Control
1 mM L-dopa
20 mV
2 sec

superfusing for about 1 minute 1mM L-dopa, the response to the diffuse stmulus decreases slightly in amplitude, indicating a reduction in the receptive field of the cell. This reduction is accompanied by an increase in input impedance. These effects are similar to the one of dopamine shown in figure 17, and were obtained in 4 other HCs. One interpretation of these results is that the metabolic pathway for dopamine production is present in the mudpuppy retina, and that the exogenously applied L-DOPA is converted into dopamine producing these effects. Obviously, there are other possibilities which should be addressed by further experimentation.

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