Synaptic Organization and Ionic Basis of On and Off Channels in Mudpuppy Retina

I. *Intracellular Analysis of Chloride-Sensitive Electrogenic Properties of Receptors, Horizontal Cells, Bipolar Cells, and Amacrine Cells*

ROBERT F. MILLER and RAMON F. DACHEUX

From the Neurosensory Laboratory, Department of Physiology, State University of New York at Buffalo, Buffalo, New York 14214

ABSTRACT Intracellular recordings from receptors, horizontal cells, bipolars, and amacrines have been carried out in the perfused mudpuppy eyecup. The introduction of a chloride-free (c-f) medium results in initial transient potential changes in many cells followed by a slow loss of light-evoked activity of the depolarizing bipolar, the horizontal cell, and the on depolarization of amacrine cells. The hyperpolarizing bipolar remains responsive to light stimulation in a c-f medium, but the antagonistic surround mechanism is abolished. These effects are reversible after returning to a normal ionic medium. The results of this study provide insight into the retinal connections which underlie ganglion cell receptive field organization. It is concluded that the depolarizing bipolar is excitatory to *on* ganglion cells and is also the pathway for on-excitation of on-off cells. The hyperpolarizing bipolar mediates the off discharge of *off* and *on-off* cells. Amacrine cells receive input from both depolarizing and hyperpolarizing bipolar cells. These findings raise the possibility that transmembrane movements of chloride ions are critical for the light responsiveness of horizontal and depolarizing bipolar cell activity.

INTRODUCTION

The neural organization of the rabbit retina is uniquely altered when the external chloride is replaced by a relatively large anion in the perfused retina-eyecup preparation (Miller and Dacheux, 1973, 1975 *a*). Multiunit ganglion cell recordings show that a chloride-free (c-f) environment results in a loss of ganglion cell discharge which follows the onset of a light stimulus, whereas the "off" discharge is well preserved. Single unit recordings have revealed that the "chloride-sensitive" ganglion cell activity includes the center and surround excitation of on-center cells, the surround excitation of off-center cells, and the on discharge component of on-off cells. A study of the c-f effects on the electro-

retinogram (ERG), proximal negative response (PNR), and horizontal cell activity suggested that at least some of the ganglion cell receptive field alterations were due to selective changes in the retinal network between photoreceptors and ganglion cells (Miller and Dacheux, 1973). In order to further evaluate this problem we have used intra- and extracellular recording techniques in the perfused mudpuppy eyecup. The findings reported here include an analysis of receptors, horizontal cells, bipolars, and amacrines. In the following paper we have examined c-f effects on the ganglion cells. The chloride-sensitive neurons have been identified as the depolarizing bipolar and the horizontal cell, an identification which provides insight into the connections between bipolars, ganglion cells, and amacrine cells as well as their intervening polarities. The third paper in this series presents a model of retinal connections consistent with the observations of this study. The fact that some neurons are sensitive to chloride replacement raises the possibility that these cells depend on chloride for light-evoked electrogenesis.

METHODS

Preparation

Mudpuppies *(Necturus maculosus)* were obtained from a dealer in the midwest and stored in refrigerated tanks containing distilled water (10°C). Experimental animals were initially decapitated and pithed. The lower jaw was removed and the head was then hemisectioned along a sagital plane. A "hemihead" section was then pinned to a special rubber holder in which a Ag/AgCl electrode was embedded. The rubber holder and the Ag/AgCl electrode were covered with a thick layer of Ringer agar. The mounted hemihead was placed under a dissecting microscope, and the cornea, iris, and lens were excised. The vitreous was drained with absorbent tissue (Kimwipe). After the eyecup was dried (approx. 30 min), the preparation was ready for the next experimental procedure. One type of experiment involved soaking the eyecup in a chloride-free solution, while the second approach used the eyecup for perfusion experiments. In the c-f soaked eyecup experiments, the hemihead was removed from the holder and submerged in a beaker of c-f Ringer and maintained there for about 1 h at 25°C. The eyecup was then drained, and mounted in a Faraday cage for recording experiments. For perfused eyecup experiments, the dried eyecup was surrounded with moist Kimwipe tissue and a "tail" of tissue, about 20 cm long, was formed to serve as a drainage channel. The preparation was then mounted in the recording cage. Small spot and annulus images were projected onto the retina, while observing with a dissecting microscope to ensure good image formation, as well as optical alignment so that the image of the small spot was centered within the annulus image. Unless otherwise indicated, the small spot was 240 μm in diameter; the annulus had an inner diameter of 600 μm and an outer diameter of 2 mm.

Perfusion

A normal and a chloride-free Ringer solution were maintained in jars resting about 3 feet above the preparation. The output of each jar was connected with a stopcock valve to a polyethylene tube which entered the preparation cage and fed to a "collecting reservoir." A single tube led from the collecting reservoir to a flow-limiting pipette which had a final tip diameter of about 0.4 mm (outer diameter). The pipette was mounted on a micromanipulator and positioned near the eyecup under microscopic observation. After initiating

fluid flow through the delivery system, the tip of the pipette was positioned adjacent to the eyecup so that the eyecup was promptly filled. The tissue surrounding the eyecup served to absorb the fluid and the "tissue tail" delivered the perfusate to a collecting well adjacent to the preparation. From the collecting well, perfusate was delivered to a beaker which rested outside the cage. Flow rate was somewhat variable and not routinely measured, but ranged between 3-6 cm^3/min.

Proper adjustment of the absorbent tissue around the eye was important. If the tissue rested far above the uppermost surface of the eye, the eyecup proved difficult to fill with the perfusate; if the tissue was considerably lower, a large convex fluid meniscus formed over the eyecup which added additional refractive power to the optical system and usually changed the spot position. Furthermore, this arrangement produced fluctuations in the fluid level and precluded stable intracellular recordings. When the tissue was properly positioned, the meniscus was reasonably flat, did not compromise the focus or spot position, and a constant eyecup fluid level was maintained. Changes in spot position (relative to an electrode tip on the retinal surface) were always checked under microscopic observation before and after initiating the perfusate. Normal perfusate consisted of the following composition: NaCl 86 mM; KCl 5 mM; $CaCl_2$ 1.8 mM; $MgCl_2$ 1.0 mM; glucose 11 mM; $NaHCO_3$ 25 mM; Na_2HPO_4 0.8 mM; NaH_2PO_4 0.1 mM. For chloride-free solutions SO_4 was usually used as a chloride substitute: Na_2SO_4 43 mM; K_2SO_4 2.5 mM; $MgSO_4$ 1.0 mM; $CaSO_4$ 6.0 mM; sucrose 45.5 mM; glucose 11.0 mM; $NaHCO_3$ 25 mM; Na_2HPO_4 0.8 mM; NaH_2PO_4 0.1 mM. A pH of 7.8 was maintained by bubbling the perfusate with 98% O_2 and 2% CO_2. The change from a normal to a c-f perfusate was accomplished by simultaneously closing the stopcock valve on the normal Ringer while opening the valve on the c-f Ringer. This was usually accomplished with little difficulty and only rarely was an intracellular recording lost because of mechanical disturbance resulting from a change of perfusate. The volume of fluid between the collecting reservoir and the eyecup was about 0.5 cm^3 and usually within 10-30 s after introducing the c-f medium, electrophysiological evidence of c-f effects was observed.

Light Stimulation

White light stimuli were provided by separate tungsten-iodine light sources (Atlas A1/215 Atlas Electric Devices Co., Chicago, Ill., color temperature supplied by manufacture 3,100°). Each source was operated at a constant current (8 A) with a regulated DC power supply. One light source provided small spot stimuli by means of a circular rotatable wheel mounted on an x-y micrometer driven base. This arrangement made it possible to change the diameter of small spot stimuli and to move spots to different retinal positions. The second light beam provided large diameter or annular light stimuli. This beam passed through a rotatable dove prism. A moving slit positioned at the plane of the aperture could then be rotated through 360° with the dove prism; this technique was used to test for motion-selective properties of ganglion cells. Both light beams were controlled by electronically regulated shutters. A collecting prism combined both beams into a single column and reflected the combined beams into the cage. A front-surfaced mirror mounted inside the cage deflected the light through a final focusing lens which was mounted above the preparation. Irradiance was measured with a Hewlett-Packard 8330A Radiant flux meter and 8334A thermopile (Hewlett-Packard Co., Avondale, Pa.), with a Fish-Schurman (#6143) heat filter (Fish-Schurman Corp., New Rochelle, N.Y.) interposed. Maximum irradiance was 4.6 mW/cm^2 for the small spot beam and 3.7 mW/cm^2 for the large spot light channel. Reduction of light intensity was accomplished by calibrated neutral density filters.

Recording

REFERENCE ELECTRODE In order to be certain that changes in the recorded potential were not due to changes in the reference electrode, the following method was used initially, and periodically throughout the course of the experiments. One amplifier (W-P 701, W-P Instruments, Inc., New Haven, Conn.) recorded between the intracellular electrode and the grounded Ag/AgCl/Ringer agar plate. A second, differential amplifier (Grass P-16, Grass Instrument Co., Quincy, Mass.) recorded between the intracellular micropipette and a second reference pipette which contacted the perfusion medium in the eyecup. Any change common to both recordings indicates that the active electrode was "seeing" the altered response. Changes in the reference electrode would be seen in the single-ended recording, but not in the differential recording. These results, based on intracellular recordings from 23 cells, showed that the initial and late c-f effects were due to changes in the active electrode. Prolonged exposure to a c-f medium (more than 1 h) did not result in a significant (more than ± 1 mV) contribution from the reference electrode system.

INTRACELLULAR Intracellular recordings were obtained with beveled glass micropipettes. Pyrex glass tubing (1.0-mm OD: 0.5-mm ID) was pulled on a Narashige vertical puller using a long heating filament. The electrodes were beveled on a rotating copper plate in which diamond dust had been embedded.[1] Beveling was done under microscopic observation. Pipettes were then filled with a conductive solution (2 M KCl or 2 M K acetate) using a back filling method. Pipette resistances measured before beveling ranged from 100–400 MΩ, and after beveling, the values were 50–250 MΩ. Electrodes were mounted on a hydraulic microdrive and cell impalement was facilitated by oscillating the high impedance amplifier (W-P M701). The amplifier also had a bridge device for passing constant current. Recordings were displayed on an oscilloscope as well as a penwriter.

A problem often encountered with micropipettes is the existence of tip potentials. Large tip potentials are commonly associated with high resistance electrodes and introduce uncertainties when making measurements of transmembrane potentials, since the magnitude of the tip potential varies with the composition of the ionic environment (Adrian, 1956; Cole and Moore, 1960). In the present study we were interested in following long-term changes in membrane potential in both a c-f and normal medium. Since the removal of external chloride ions probably causes intracellular changes in chloride concentration (Hodgkin and Horowicz, 1959), we were interested in determining the effects these changes might have on the tip potential and whether the technique of beveling reduced tip potentials and rendered the electrode less sensitive to changes in the ionic environment. The tip potentials of 35 electrodes were studied. Twenty electrodes were filled with KCl and fifteen were filled with K acetate. Tip potential changes were evaluated in solution of high K^+ (86 mM) while the chloride concentration varied from 95 to 52 mM to 0 using propionate or sulfate as a chloride substitute. Each electrode was evaluated before and after beveling and final tip potential changes were determined by subtracting the values (in each solution) obtained after breaking the tip. Broken-tipped electrodes were insensitive to changes in external chloride. Although electrode beveling always reduced electrode resistance, little if any change in tip potential was observed and no significant differences were noted in tip potential changes to chloride replacement. Our observations suggest that if internal chloride concentration changed from 95 mM to 0, a change in tip potential of -5 to -7 mV would occur. Thus changes in intracellular chloride concentration can have an effect on tip potential, and the magnitude of this effect is not improved by the beveling. The change in tip potential will depend on several

[1] We are indebted to Dr. Michael Behbehani for bringing this method to our attention.

factors, among which will be the change in internal chloride concentration which may occur during an exposure to a c-f environment. We have adopted the general view that rapidly occurring changes in potential which accompany the introduction of a c-f medium are true changes in transmembrane potential (Motokizawa et al., 1969), whereas slower changes, particularly slow hyperpolarizations of approximately a 5-mV range, could result from tip potential changes induced by a loss of intracellular chloride.

The major difficulty encountered in this study relates to the necessity for long-term intracellular recordings. In order to see the c-f effects on retinal neurons, as well as recovery after returning to a control medium, intracellular recordings had to be maintained for periods of 5–30 min. It was particularly difficult to maintain bipolar, receptor, and amacrine cell recordings for prolonged periods of time. Nevertheless the cells reported here only include neurons from which recordings were maintained long enough to see the c-f effect as well as either complete or partial recovery. Obviously, the actual number of cells from which partial data were obtained far exceeds the number reported here. Zestful youth, lion-heartedness, and an indifference to unsuccessful experiments seemed to be helpful attributes for this type of experimental approach.

One hundred and seventy-one cells were studied, including 13 receptors, 67 horizontal cells, 23 depolarizing bipolars, 37 hyperpolarizing bipolars, and 14 amacrine cells. Each cell type was identified according to the physiological criteria established by Werblin and Dowling (1969); however, 17 cells could not be clearly identified. Responses were displayed on a storage oscilloscope and a penwriter. Figures were reproduced from Polaroid photographs of the storage screen, or more commonly from photographic reproduction of penwriter records. Because a long exposure to the c-f medium was required, only sections of the records were reproduced in each figure. The sections are displaced vertically according to the observed change in recorded potential.

RESULTS

Receptors

Receptors were relatively insensitive to the removal of external chloride. Fig. 1 (lower left-hand section) illustrates a receptor recording in a normal Ringer perfusate. The middle section shows the same receptor after 6 min in a c-f environment. The right-hand section illustrates the response observed 1 min after returning to the control Ringer solution. Receptors were identified by the relative depth of recording and similar amplitude responses to small or large diameter light stimuli. An additional technique was employed with four receptors to further identify the receptor origin of the recording. A Ringer solution containing 20 mM $MgCl_2$ and 0.5 mM $CaCl_2$ perfused the retina for 4–6 min. When applied to postreceptor neurons, this solution resulted in a decreased response amplitude, but in the receptors, a slight increase in amplitude was usually observed. Though not illustrated, the high Mg^{++} solution was applied to the receptor recording shown in Fig. 1 and resulted in an increased amplitude of 15% accompanied by a depolarizing off response of about 1 mV. After the exposure to Mg^{++}, a c-f medium was introduced and the results are illustrated in Fig. 1. The removal of external chloride did not result in a change in membrane potential but a slight decrease (10%) in response amplitude was observed. A small depolarizing off response of the receptors was also seen in the c-f environment. On returning to the control, the response was further reduced perhaps indicating some deterioration of the recording. The insert in Fig. 1, obtained

from another receptor, shows superimposed traces recorded in the normal medium and 3 and 4 min after introducing a c-f Ringer. In this case the c-f environment resulted in a 25% increase in amplitude and the appearance of a sizable depolarizing off response, similar in appearance to the off response induced by high Mg^{++}. In most receptors the presence of a depolarizing off response was observed in a c-f medium. In 2 of the 13 receptors studied, the c-f medium resulted in a transient depolarization followed by a rapid return to the original base line. During the depolarization, light stimuli usually did not evoke a response or responses were reduced in amplitude. The failure to see an early depolarization and "silent period" in other receptors may reflect differences in

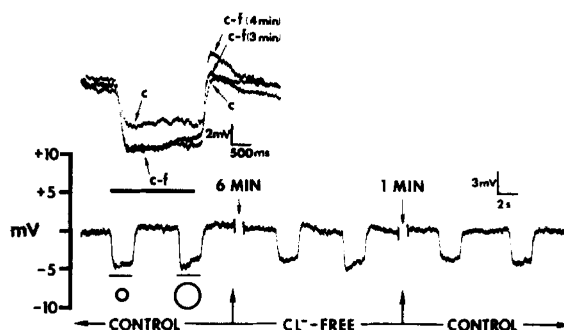

FIGURE 1. C-f effects on intracellularly recorded receptors in perfused mudpuppy eyecup preparation. Responses on left were elicited by small spot and diffuse light stimulation in control Ringer. Middle section recorded 6 min after initiating c-f medium. Responses were slightly smaller and small off depolarization was seen. Responses on right were recorded 1 min after returning to normal Ringer showing slight reduction in response amplitude. Insert shows c-f effects on another receptor. Superimposed traces were elicited in control Ringer *(c)*, 3 and 4 min after initiating a c-f Ringer; all responses elicited by diffuse light stimulation. Note slight depolarization of recording observed in c-f responses. C-f medium resulted in increase in response amplitude (25%) and appearance of depolarizing off response. Irradiance: small spot 4.6×10^{-7} W/cm^2; diffuse 3.7×10^{-7} W/cm^2.

chloride permeability or could alternatively reflect variations in the efficiency of removing chloride. If external chloride was slowly reduced in concentration, transmembrane chloride movement would be less apparent than if a rapid, uniform reduction was achieved.

In one receptor recording a 0.5-s (5×10^{-10} A) intermittent current pulse was applied to the recording electrode after balancing the bridge circuit. During the exposure to the c-f environment, changes in input resistance were evaluated. No change in the input resistance of the cell was observed during a 6-min exposure to the c-f environment. These findings, consistent with those of other workers (Miller and Dacheux, 1973; Winkler, 1973; Cervetto and Piccolino, 1974; and Brown and Pinto, 1974), show that chloride is not a critical ion for receptor electrogenesis.

Horizontal Cells

Fig. 2 illustrates the slow loss of light-evoked activity of a horizontal cell during an exposure to a c-f environment. In our experience these cells generally have about a −30-mV dark membrane potential when measured with low resistance (20 MΩ), beveled micropipettes; this value is indicated on the left. The responses on the left show the principle identifying characteristics of horizontal cell responses. A small spot stimulus (right response) evoked a smaller hyperpolarization than a diffuse light stimulus. Other features of these cells include a relatively slow rise time and a relatively sustained hyperpolarizing response. The middle section of Fig. 2 shows the behavior of the horizontal cell during an exposure to the c-f environment. The responses in the middle and right-hand section were recorded at a slower speed than those on the left, and were evoked

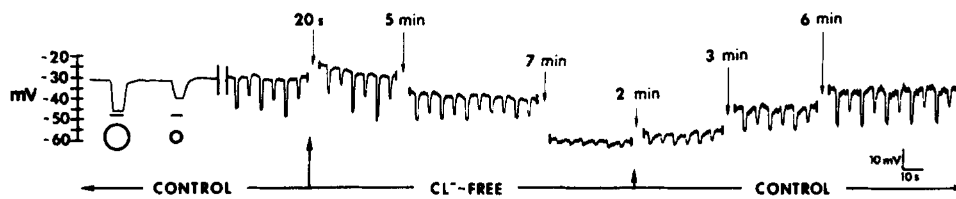

FIGURE 2. C-f effects on horizontal cell activity. Left-hand section shows different amplitude responses to small spot and diffuse light stimulation. Remaining responses recorded at reduced speed, alternating small spot and diffuse light stimulation. C-f perfusate resulted in an early depolarization and increased response amplitude, followed by slow hyperpolarization of cell. After 5-min 20-s responses to diffuse and small spot stimulation were nearly equal in amplitude. After 12 min 20 s no slow hyperpolarization was evident and responses were small and irregular; cell had become hyperpolarized to about −60 mV. Returning to control Ringer did not elicit early transient changes in potential, but slow depolarization was accompanied by gradual recovery of light-evoked responses. Irradiance: small spot 4.6×10^{-7} W/cm^2; diffuse light 3.7×10^{-7} W/cm^2.

by alternating small spot and diffuse light stimuli. Soon after initiating the c-f perfusate, the horizontal cell was slightly depolarized and the light-evoked responses were larger than the control values. The cell gradually hyperpolarized as the light-evoked responses decreased in amplitude. After 5 min the response amplitude to the small spot and diffuse stimuli were nearly equal; at 12 min, 20 s, the hyperpolarizing phase had ended, light-evoked responses were equal, and the cell had become hyperpolarized to about −60 mV. On returning to the control Ringer, the process was reversed with recovery of light-evoked responses accompanied by depolarization of the cell. Recovery required about the same amount of time as that required to see a steady-state, c-f condition. In 14 cells a brief (0.5×10^{-9} A) intermittent current pulse was passed through the recording electrode after balancing the bridge device. The relative change in input resistance (electrode resistance and cell resistance) was monitored by continually readjusting the bridge balance and reading the resistance value from a calibrated bridge potentiometer. In 12 cells a progressive increase in input resistance was

observed as the cell hyperpolarized during the c-f effect. Final resistance values in the steady-state c-f condition were two to three times the values observed in the control. A return to the normal perfusate was accompanied by a gradual decrease in input resistance. Two cells showed a slight decrease in input resistance during the c-f effect. However, both cells showed only a minor hyperpolarization in the c-f medium and depolarized to small amplitude responses on returning to the control, suggestive of deterioration of the cell. All but four cells showed an initial, c-f-induced depolarization followed by an early increased amplitude phase before the period of slow hyperpolarization was evident. Thirteen cells also showed a silent period which occurred early in the depolarization phase, followed by a later period of increased light-evoked response amplitude. On returning to the control environment after a steady-state c-f response, no early transient changes in potential were observed in most cells. Three cells showed a slight hyperpolarization as an early response to the normal ionic medium.

Bipolar Cells

DEPOLARIZING BIPOLARS (DPB) The depolarizing bipolar cells (DPB) like the horizontal cells were sensitive to the removal of external chloride ions. However, unlike the horizontal cells, the loss of the light-evoked response in the bipolar was not accompanied by any large hyperpolarization. Fig. 3 shows the identifying characteristics of a depolarizing bipolar as well as the effects of an exposure to a c-f environment. The small spot light stimulus resulted in a peak depolarization followed by a sustained response, whereas flashing an annulus produced a small depolarization, followed by a return to the base line and a

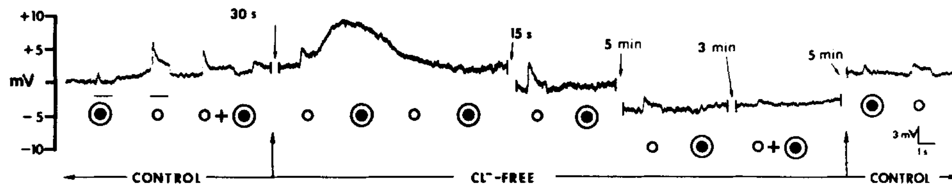

FIGURE 3. C-f effects on depolarizing bipolar. Small spot stimulus evoked transient and sustained responses in control medium (left-hand section); annulus evoked small on and off depolarizations. Annulus stimulation concentric with steady small spot illumination evoked a hyperpolarizing response thereby demonstrating center-surround antagonistic organization of cell. A transient depolarization occurred 30 s after initiating a c-f perfusate. Transient depolarization accompanied and followed by a silent period during which light stimulation did not evoke response; recovery from silent period was evident 15 s later. After 5 min 45 s response amplitude was decreased and cell had become hyperpolarized by about 4 mV compared to control. Response to annulus was almost abolished. An additional 3 min showed small response to small spot stimulus but spot plus annulus gave no response. Traces on right show some recovery of light-evoked response after returning to control environment. Irradiance: small spot 4.6×10^{-7} W/cm^2; annulus 3.7×10^{-7} W/cm^2.

small off response. However, if the small spot was turned on continuously, the annulus flash produced a hyperpolarizing response thereby demonstrating the antagonistic center-surround organization of the cell. Thirty seconds after initiating the c-f perfusate, a rapid 5-mV depolarization occurred during which the cell did not respond to light stimulation. This silent period lasted for about 20 s after which the light-evoked responses returned. Further exposure to the c-f medium resulted in a slow hyperpolarization and a decrease in response amplitude. The response to the annulus was abolished before the response to the small spot stimulus, and after 8 min, 45 s the small spot plus annulus did not produce a hyperpolarization. A return to normal Ringer did not produce transient changes in potential; rather a slow depolarization was accompanied by a gradual recovery of the response amplitude. The right-hand portion of Fig. 3 shows that the response recovered to about one-third of the control amplitude after 5 min into the normal perfusate. Conductance measurements show that the light-evoked depolarization is accompanied by an increased conductance. In the c-f medium, at the time of response loss, light stimuli did not produce a conductance change. Recovery of the response in a normal medium was accompanied by a return of the light-mediated change in conductance.

HYPERPOLARIZING BIPOLAR (HPB) HPB cells were relatively insensitive to the removal of chloride ions, but their antagonistic surround was inverted to a hyperpolarizing response by this procedure. Fig. 4 illustrates a HPB. The left-hand trace shows the response to a small spot stimulus which evoked a sustained hyperpolarization; a diffuse light stimulus produced a large amplitude response which consisted of a transient as well as sustained component. When the small spot stimulus was turned on continuously, a concentric annulus flash resulted in

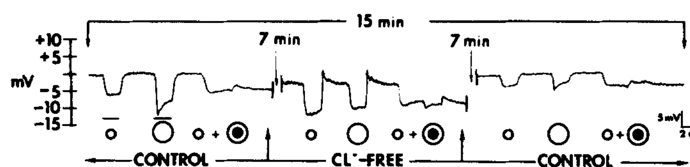

FIGURE 4. C-f effects on hyperpolarizing bipolar. Responses on left obtained in control Ringer. Small spot stimulus evoked sustained hyperpolarization; diffuse stimulus evoked transient and sustained response. Annulus stimulus concentric with steady small spot illumination resulted in a depolarization demonstrating the antagonistic center-surround organization of the cell. Middle section recorded 7 min after initiating c-f perfusate. Response to small spot stimulus slightly larger than diffuse stimulus response. Annulus superimposed on steady small spot illumination evoked hyperpolarizing response indicating loss of antagonistic surround mechanism. Presumably the annulus-evoked hyperpolarization was due to additional center-mediated hyperpolarization. Section on right shows response observed 7 min after returning to control Ringer. Response amplitudes decreased. Small spot plus annulus elicited depolarizing response thereby demonstrating return of antagonistic surround mechanism. Irradiance: small spot 4.6×10^{-7} W/cm^2; diffuse stimulus and annulus 3.7×10^{-7} W/cm^2.

a depolarizing response, revealing the antagonistic center-surround organization of the cell. The middle section of Fig. 4 was recorded after 7 min of perfusion with a c-f medium and shows that the responses to small spot and diffuse light stimulation were nearly equal in amplitude and a depolarizing off response was apparent. When the small spot was turned on continuously, the annulus flash resulted in a hyperpolarization. Presumably the hyperpolarization elicited by the annulus was due to additional stimulation of the center mechanism. The responses on the right, recorded 7 min after returning to the control Ringer, were smaller and no off depolarization was present. The small spot plus annulus stimulation resulted in a depolarizing response indicating that the antagonistic surround mechanism had been reestablished.

Hyperpolarizing bipolars often showed an early depolarization and a silent period similar to that observed in DPB cell recordings. In steady-state c-f conditions, HPB cells were usually slightly hyperpolarized by a few millivolts, and then depolarized slightly after returning to the control medium. In some HPB cells the relative change in input resistance was evaluated using the bridge balance technique. In general, a c-f medium resulted in an increase in input resistance, but these changes were less than those observed in the horizontal cell. It is thus possible that chloride ions make some contribution to the resting conductance of HPB cells, but clearly chloride does not play a major role in the center-mediated hyperpolarization of these cells.

AMACRINE CELLS Amacrine cells are the most distal retinal neuron in which impulse activity has been recorded. In the mudpuppy the only amacrine cell type identified with certainty is the on-off cell (Werblin and Dowling, 1969). This raises a question of how amacrine cells are distinguished from on-off ganglion cells. We have studied this problem extensively in freshly excised perfused, and nonperfused eyecup preparations. Separation between the two cell types is not difficult and can be made on the basis of impulse activity as well as excitatory-inhibitory postsynaptic potential (EPSP-IPSP) responses. A more complete description of these findings is presented in the following paper. In summary, amacrine cells generate two different types of impulses which are probably of dendritic and somatic origin; only a single type of impulse is observed in ganglion cells. Another important distinction between the two cell types is the nature of the slow potential which remains after the impulse mechanisms have been abolished due to injury depolarization. On-off ganglion cells show an on and off EPSP-IPSP sequence, whereas amacrine cells show only an on and off EPSP, with EPSP amplitudes considerably larger (4–30 mV) than those observed in on-off ganglion cell recordings (1–5 mV). Other differences, such as the accommodation levels of spike-generating mechanisms, are discussed in the following paper. Of the 13 amacrine cells studied, 7 showed evidence of both somatic and dendritic spike activity immediately after cell penetration; 4 cells showed only somatic spike activity and 2 showed only on and off EPSP responses. In all cases, however, the long-term recordings required for this study eventually led to a recording condition in which impulse activity was inactivated, leaving EPS responses.

Fig. 5 illustrates the c-f effects on an intracellularly recorded amacrine cell. A diffuse light stimulus was used throughout this study. In a control medium (left-hand section) the light stimulus resulted in transient on and off depolarizations. The introduction of a c-f medium resulted in a depolarization of about 10 mV during which the cell was unresponsive to light stimulation. The left middle section was recorded after a 1-min c-f exposure and shows that the on and off responses were about the same amplitude as in the control, but the declining phases were more prolonged. After 4 min of c-f exposure (right middle section), the on depolarization was abolished but the off response was about the same amplitude as the control off response. The decay phase of the off response was shorter than the decay phase of the earlier c-f recording. Recovery of the on depolarization was evident within 2 min after returning to the normal Ringer solution, though both on and off responses were smaller than those in the initial control. Amacrine cells were initially depolarized in the c-f medium, though

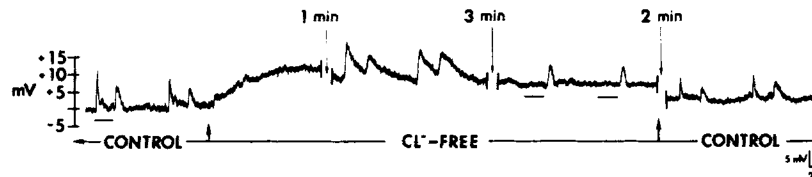

FIGURE 5. C-f effects on amacrine cell. Diffuse light stimulation in control Ringer (left-hand section) evoked on and off transient depolarizing response, but no evidence of on-off IPSP's. C-f medium produced depolarization and silent period; 1 min later on and off depolarizations had returned. After 4 min in a c-f medium on response was abolished but off responses remained. Note decay phase of off response after 4 min is faster than response after 1 min in c-f medium. Two minutes after returning to control Ringer, cell had returned close to original membrane potential and on and off depolarizations were evident. Irradiance: diffuse light stimulation 3.7×10^{-7} W/cm^2.

further exposure usually resulted in a gradual hyperpolarization. In a steady-state c-f condition some amacrine cells were slightly depolarized and some were slightly hyperpolarized compared to the original recorded potential. In a few cells, especially those of large amplitude, the loss of the on depolarization unmasked a small sustained hyperpolarization during the light stimulus (see Fig. 6).

UNIDENTIFIED CELLS Seventeen cells could not be clearly identified by the criteria of Werblin and Dowling (1969). Six of these cells were depolarizing and 11 were hyperpolarizing units. It was not clear whether the depolarizing cells constituted a single "class," but in general these units appeared bipolar-like but did not have antagonistic surrounds when tested with the center plus annulus technique. Likely sources of these responses include ganglion cells, amacrine cells, or bipolars. In all cases, however, a c-f environment resulted in a loss of their light-evoked activity. Some depolarizing units showed a prominent hyper-polarizing transient at the termination of the light stimulus. This hyperpolariza-

tion was immediately inverted to a depolarizing response in a c-f environment, consistent with the behavior of chloride-dependent IPSP responses (Miller and Dacheux, 1976). Also, intracellular chloride injections with KCl electrodes were effective in inverting these responses.

Eleven hyperpolarizing units could not be identified. These cells showed a fairly large amplitude response to a small spot of light, and a slightly larger response to a diffuse light stimulus. The responses had faster rise times than horizontal cells and were relatively sustained with no evidence of antagonistic organization. They had a similar appearance to hyperpolarizing bipolar cells and were not abolished in a chloride-free medium.

In summary, our findings suggest that depolarizing bipolar cells, the horizontal cell responses, and the depolarizing on response of the amacrine cells are abolished in a c-f environment. Further support of these findings has been obtained from intracellular recording experiments, carried out on c-f-soaked eyecups. Soaking the eyecup in a c-f medium for 1 h results in a characteristic, long-lasting loss of on ganglion cell discharge. One hundred and thirty cells were examined with this technique, and in every case the observations made in the perfused eyecup were confirmed: no on depolarizing responses were observed, and the remaining light-sensitive cells included receptors, hyperpolarizing bipolars, depolarizing off responses of amacrine cells, and off impulse activity of ganglion cells.

Fig. 6 summarizes the chloride-sensitive and insensitive neurons studied in the mudpuppy eyecup. The responses on the left show the different cell types and some organizational features observed in the normal eyecup. The right-hand column illustrates the cells which remain responsive to light stimulation in a c-f environment.

DISCUSSION

The results of this study pose two major questions. First, what information about interneuronal connections and polarities is revealed by the selective effects of a c-f environment, and second, what is the mechanism by which removing external chloride abolishes the light responsiveness of the chloride-sensitive neurons?

Interneuronal Connections

In a c-f environment, the hyperpolarizing bipolar is the only interconnecting cell between receptors and neurons of the inner retina. Thus the hyperpolarizing bipolar must subserve the ganglion cell discharge which remains responsive to light stimulation in a c-f medium. This includes the off discharge of off-center, and on-off ganglion cells (Miller and Dacheux, 1975 *a*, 1976). By elimination, the on discharge of on-center and on-off cells must be subserved by the depolarizing bipolar. The identification of the chloride-sensitive neurons, together with the experimental findings of the following paper have been incorporated into a model which describes the retinal connections and polarities underlying the three types of ganglion cells (on-center, off-center, and on-off). This model is described in the third paper of this series.

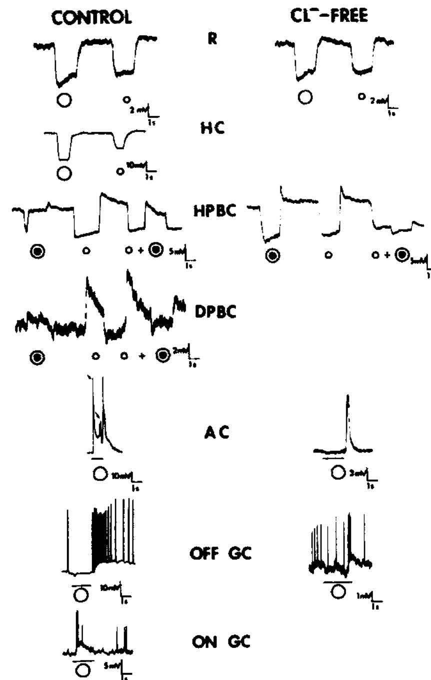

FIGURE 6. Summary diagram of chloride-sensitive and insensitive retinal neurons. Left-hand column shows responses observed in control Ringer; right-hand column shows responses obtained in a c-f medium. *Receptors* (R) are relatively unaffected by c-f environment. *Horizontal cells* (HC) become insensitive to light stimulation in a c-f medium as cell is hyperpolarized. *Hyperpolarizing bipolar cells* show antagonistic center-surround organization in control, but surround mechanism is abolished in c-f medium, leaving only center-mediated hyperpolarization. *Depolarizing bipolar cells* show antagonistic center-surround organization in control but lose both center and surround responses in a c-f medium. *Amacrine cells* show on and off somatic spikes (first arrow) followed by smaller dendritic spikes (second arrow) in control Ringer. Some amacrine cells show light-evoked hyperpolarization in c-f medium accompanied by loss of on depolarization. Amacrine cell on right recorded from a c-f-soaked eyecup preparation. *Ganglion cells.* On and off ganglion cells evident in control environment. Off impulse activity shown in on cell represents spontaneous activity resulting from cell penetration. Only off responding cells are observed in a c-f medium. Note off cell in c-f shows light-evoked hyperpolarization preceding off discharge.

Chloride-Free Mechanisms

A second question posed by these findings concerns the mechanism by which a c-f environment selectively alters the retinal network. The chloride-sensitive neurons include the depolarizing bipolar, the horizontal cell, and the postsynaptic responses and ganglion cell mechanisms which they subserve. Certainly one possible explanation of our findings is that transmembrane movements of chloride ions are critical for the electrogenesis of the chloride-sensitive neurons.

However, other possible mechanisms must also be considered and are briefly discussed below.

ANION SUBSTITUTES The c-f effects described in this study are not due, specifically, to the presence of sulfate which was the most commonly used chloride substitute in these experiments. Methylsulfate and propionate produce identical results, and bromide substitutes well for chloride without any observed alterations in ganglion cell discharge (Miller and Dacheux, 1973).

CHLORIDE-FREE EFFECTS ON CATIONS In frog muscle, replacing chloride with an impermeant anion results in a depolarization of the cell due to the high chloride conductance and the initial outward movement of chloride ions (Hodgkin and Horowicz, 1959). The depolarization induces outward K^+ movements and the original membrane potential is eventually restored after KCl and water have moved out of the cell with the final intracellular concentration of chloride reduced and that of K^+ unchanged. If retinal neurons have a similar K^+ and Cl^- permeability, the cell shrinkage could result in an increased intracellular Na concentration and Na^+-dependent responses would be reduced in amplitude. However, if the cell were permeable to both K^+, Na^+, and Cl^-, and the Na^+ permeability were not insignificant as is the case in frog muscle, then outward Na^+ movement could also occur in a c-f environment and intracellular Na^+ concentration changes would be less.

In the present study the initial effects of a c-f solution included a transient depolarization accompanied by a silent period during which light stimulation either did not evoke a response, or responses were reduced in amplitude. Ganglion cell recordings also show a similar sequence of events during the early c-f experience (Miller and Dacheux, 1976). These observations cannot be interpreted as an indication of chloride permeability for any particular cell. The depolarization could be due to: (*a*) outward Cl^- movement, (*b*) synaptically mediated depolarization, or a third possibility might involve K^+ movements. An outward movement of chloride could induce an outward movement of K^+, and if this were of sufficient magnitude an increase in external K^+ might result and lead to depolarization of neighboring cells. The fact that at least some receptors experienced a silent period suggests that this phenomenon may involve c-f effects on the receptors themselves. The relatively brief duration of the silent period probably reflects a rapid redistribution of chloride ions, or a rapid return of increased external K^+, or both.

CHLORIDE-FREE EFFECTS ON ANIONS An additional mechanism involves an anion exchange system and its possible effect on intracellular pH. In this case the outward chloride movements would induce inward movement of another anion, and if this were the exclusive exchange, no cell shrinkage would result. The only anion in significant concentration in our perfusate is HCO_3^-. The entrance of significant quantities of this ion into the intracellular compartment could result in titration of cell buffers in the alkaline direction and possibly affect intracellular structures which are involved in conductance mechanisms of other ionic channels. In frog muscle, HCO_3^- conductance has been estimated at about 0.1 that of chloride (Woodbury and Miles, 1973). Furthermore, HCO_3^- injections

into spinal motoneurons show that this anion does not substitute for chloride (Coombs et al., 1955). Thus HCO_3^- is a relatively impermeant anion for both the resting chloride channels of frog muscle, and the synaptically activated chloride channels of motoneurons. If these results can be applied to retinal neurons, it is unlikely that large quantities of HCO_3^- movements would occur but it is difficult to evaluate the significance of small movements.

MEMBRANE RESISTANCE Replacing chloride with an impermeant anion will increase the membrane resistance of those cells which have some resting chloride conductance. An increase in membrane resistance can magnify the contribution of an electrogenic pump to the membrane potential (Carpenter, 1973; Hertog, 1973). The c-f-induced hyperpolarization observer in horizontal cell recordings could be due to a hyperpolarization of the subsynaptic membrane by an electrogenic pump mechanism and the pump activity could be stimulated by an increase in intracellular sodium. It seems unlikely that his mechanism can account for all of our findings but additional experiments will be necessary to evaluate this possibility.

CHLORIDE-DEPENDENT ELECTROGENESIS Studies of several perfused neuronal systems (Kerkut and Thomas, 1964; Takeuchi and Takeuchi, 1967; Barker and Nicoll, 1973) indicate that a c-f perfusate does not seriously compromise neuronal activity and synaptic interactions which do not depend on chloride for their electrogenesis. Under c-f conditions, impulse activity, synaptic transmission, and EPS responses remain, and only chloride-dependent potentials are abolished. This raises the possibility that a c-f medium offers a relatively simple condition in which chloride-dependent responses can be separated from those which do not depend on this ion. This is a particularly intriguing possibility when one considers the highly selective nature of a c-f environment on retinal neurons. It is thus worth taking into account the behavior of chloride-dependent responses in a c-f environment.

CHLORIDE-DEPENDENT IPSP'S Chloride-dependent IPSP's have been described in a number of neurons (Kerkut and Thomas, 1964; Takeuchi and Takeuchi, 1967; Barker and Nicoll, 1973) in which external perfusion has been carried out. These hyperpolarizing responses are inverted to depolarizing responses during the initial c-f exposure. The c-f environment shifts the chloride equilibrium potential to a positive value, and an increase in chloride permeability results in an outward movement of chloride ions. A prolonged c-f exposure results in a gradual decline in the depolarizing response as chloride is "washed out" of the cell. In the following paper we demonstrate this type of behavior for a chloride-dependent IPSP observed in on-off ganglion cells. Also, in the present study, a few unidentified depolarizing cells had a hyperpolarizing "off transient" which was quickly inverted to a depolarization during the early exposure to a c-f medium. However, apart from these two responses, it is clear that none of the retinal neurons behave in this way, and we conclude that chloride-dependent IPS mechanisms are not the dominant mode of synaptic interaction in the retina.

The findings of this study demonstrate that both depolarizing and hyperpo-

larizing responses are abolished in a c-f medium. On the other hand, it is also apparent that some depolarizing and hyperpolarizing responses persist in a c-f perfusate. Two general principles are relevant to this problem. First, an increase in chloride permeability can lead to a hyperpolarization or a depolarization depending on the internal concentration of chloride with respect to the membrane potential (Oomura et al., 1965). Second, a hyperpolarizing response can result from inhibition or disfacilitation; a depolarizing response can result from excitation or disinhibition. In the following section these principles are applied in evaluating the c-f effects on each of the retinal neurons. For any cell, the central problem is separating a direct c-f effect from c-f actions on other neurons which modify the input to that cell.

Receptors In a number of vertebrate receptors, electrogenesis has been shown to be relatively insensitive to the removal of chloride ions (Miller and Dacheux 1973; Winkler, 1973; Cervetto and Piccolino, 1974; Brown and Pinto, 1974). The outer segments of frogs have a high sodium permeability in the dark and the inner segments and synaptic regions presumably have a high K^+ permeability (Sillman et al., 1969; Korenbrot and Cone, 1972). Since the action of light is to decrease sodium permeability, the resulting hyperpolarization can be viewed as a disfacilitatory type of response. This does not mean, however, that chloride ions do not play some role in receptor function. A feedback mechanism from horizontal cells to cones has been demonstrated in receptors of the turtle (Baylor et al., 1971) and Gecko (Kleinschmidt, 1973; Pinto and Pak, 1974). O'Bryan (1973) has shown that at least one component of this feedback is accompanied by an increase in conductance. It is possible that this mechanism is Cl^- dependent. In the present study we observed a consistent off-depolarization in receptors in a c-f medium. This often occurred without any detectable change in membrane potential. The increased off response in a c-f environment has no obvious explanation; but, it could be related to a loss of feedback from the chloride-sensitive horizontal cells. It is noteworthy that a perfusate (20 mM Mg, 0.5 mM Ca^{++}) which reduces or blocks postreceptor neuronal activity results in an off response in receptors which resembles that seen in a c-f medium.

Horizontal Cells Horizontal cells respond to light with a hyperpolarization. In many cases the hyperpolarization is accompanied by a decrease in conductance (Trifonov, 1968; Trifonov and Byzov, 1965; Toyoda et al., 1969). Trifonov (1968) explained this finding by suggesting that the receptors release a depolarizing transmitter in the dark and that the effect of light was to reduce the amount of transmitter released, thereby causing a hyperpolarization of the cell (i.e., disfacilitation). In the skate, Dowling and Ripps (1973) have supported this view by showing that an increase in external Mg^{++} results in a hyperpolarization of the horizontal cell and a loss of the light-evoked response. We have recently confirmed these observations in the mudpuppy (Dacheux and Miller, 1976). According to this model, the subsynaptic membrane is depolarized in the dark, but the nonsynaptic membrane is permeable to other ions, perhaps K^+. What ions are involved in the dark-mediated depolarization? One possibility is that an

increased Na^+ permeability underlies the dark-mediated depolarization. A second possibility is that an increased chloride conductance exists and that horizontal cells maintain internal chloride at a relatively high concentration such that the chloride equilibrium potential is more positive than the K^+ equilibrium potential of the nonsynaptic membrane. Consistent with the latter idea are some of the transient effects of the c-f medium on the horizontal cell. According to the "chloride hypothesis" the initial removal of chloride ions should shift the chloride equilibrium potential to a more positive value causing a depolarization as chloride ions moved out of the cell. This depolarization would occur primarily at the subsynaptic membrane and during this period the response amplitude should be increased: these results were observed in the majority of cells. Then as chloride washes out of the cell and is reduced in concentration, the membrane potential will be more dominated by the equilibrium potential of the nonsynaptic membrane (i.e., the K^+ equilibrium potential). The large increase in input resistance during the chloride "wash-out" period is consistent with a steady loss of internal chloride and a reduction in the available anion to move through the synaptically opened channels. On returning to the control environment, an initial hyperpolarization might be expected as chloride reentered the cell; this was observed in only three cases. However, because the cell is markedly hyperpolarized, the inward chloride-induced negativity might be too small for easy detection. More recently we have examined the effects of substituting different fractions of the external chloride; in most cases, the reintroduction of a normal chloride concentration resulted in an initial hyperpolarization during which light-evoked responses were reduced in amplitude. Presumably, as soon as Cl^- was passively distributed according to the membrane potential, an inward pump would be required to increase intracellular chloride and reestablish the dark membrane potential. An inwardly directed chloride pump has been established in squid (Keynes, 1963).

Recently Waloga (1975) has studied horizontal cells in the perfused axolotl retina. A low sodium perfusate produced a greater attenuation of horizontal cell responses when compared to receptors. By using a background light to reduce the receptor response to an equivalent reduction observed in the low sodium environment, she demonstrated that horizontal cell responses were only minimally reduced in amplitude. From these experiments she argued that the low sodium effects on horizontal cells were not due to nonlinearities in receptor-horizontal cell transmission, but indicate a sodium sensitivity of the horizontal cell in addition to the known sodium dependency of receptors. The horizontal cell responses in axolotl retina are also abolished in a c-f medium. However in the axolotl cells the c-f effects did not include an early depolarization, enhancement phase, and in this respect differ from the observations of this study. Waloga has suggested that the principle action of the receptor transmitter is to increase sodium permeability.

Nelson (1973) has studied horizontal cells in the mudpuppy using intracellular ion injection. With NaCl-filled electrodes, the injection of Na^+ ions resulted in an increase in response amplitude in most cases. He argued that this experiment was not consistent with the idea that Na^+ was an important ion for maintaining

the dark membrane potential of the horizontal cell since an intracellular Na^+ would result in a decrease in response amplitude. However, positive current injection could also lead to an increase in intracellular chloride, particularly in those regions of the cell where a high chloride permeability existed (Coombs et al., 1955). This would depolarize the cell and the light-evoked amplitude would increase according to the above chloride hypothesis. It is clear that more work will be required before the c-f and low sodium effects on horizontal cells are understood. It should also be stated that the chloride hypothesis and "sodium hypothesis" are not mutually exclusive, and that both ions might be involved in dark-mediated permeability changes.

Hyperpolarizing Bipolars Hyperpolarizing bipolars, like the horizontal cell, undergo a conductance decrease in response to light stimulation (Nelson, 1973; Toyoda, 1973). From this observation it has been suggested that the response of these cells is generated in a manner similar to the horizontal cell response. Our observations of hyperpolarizing bipolars, using synaptic blocking agents, are consistent with this view (Dacheux and Miller, 1976). However the results of this study show that the removal of chloride clearly differentiates between horizontal cells and hyperpolarizing bipolars. Thus as chloride can be implicated as a possible ion for horizontal cell electrogenesis, it clearly plays an insignificant role in the electrogenesis of the hyperpolarizing bipolar. Certainly an alternative possibility is that the hyperpolarizing bipolar depends on a high Na conductance in the dark, and like the horizontal cell, the nonsynaptic region could be a K^+-dependent membrane.

A c-f environment results in a loss of the antagonistic surround of hyperpolarizing bipolars. Other workers have suggested that this mechanism is mediated by the horizontal cells (Werblin and Dowling, 1969, Kaneko, 1970). This influence is apparently mediated by a feedback mechanism onto receptors (Baylor et al., 1971), or through a direct influence on bipolar cells (Dowling and Werblin, 1969), or both. The loss of the horizontal response in a c-f medium is thus sufficient to account for the loss of the antagonistic organization of these cells. These experiments, however, do not eliminate the possibility that changes in chloride conductance at the level of the bipolar cell are necessary for surround antagonism.

Depolarizing Bipolars Unlike the horizontal cell and hyperpolarizing bipolar, depolarizing bipolar cells experience a conductance increase to light stimulation (Toyoda, 1973; Nelson, 1973). Thus one would argue that these cells behave as though an excitatory transmitter were released in the light as opposed to the dark released excitatory transmitter proposed for horizontal cell and HPB cell generation. Recently, we reported some experiments which help to clarify this issue (Dacheux and Miller, 1976). In the perfused mudpuppy eyecup we added cobalt (2 mM) to serve as a synaptic blocking agent. Intracellular recordings from hyperpolarizing bipolars and horizontal cells show that a brief (1–2 min) exposure to 2 mM cobalt resulted in a loss of light-evoked activity accompanied by a large hyperpolarization of these cells. Depolarizing bipolars on the other hand were depolarized by the action of cobalt. These findings are thus consistent

with the idea that receptors release a transmitter in the dark and that it has a depolarizing action on horizontal cells and hyperpolarizing bipolar cells, but a hyperpolarizing influence on the depolarizing bipolars. One possible explanation of our findings is that a dark-released transmitter decreases the conductance of chloride ions, and that the internal chloride concentration is relatively high. The transient c-f effects are consistent with such a model. According to this view a c-f environment would have minor effects on the membrane potential, but a progressive decrease in amplitude (preceded by an early enhancement phase) would occur as the chloride washed out of the cell. This is in fact the observed behavior of depolarizing bipolar cells.

A synaptically mediated hyperpolarization accompanied by a decreased conductance has been described in the frog sympathetic ganglion (Weight, 1974). However, this mechanism has a long latency and duration of action, and presumably operates by an intracellularly mediated "messenger." It is unlikely that this type of synaptic action would be positioned in a relay for which speed is important. On the other hand, a transmitter which decreased a high conductance without an intervening intracellular relay might account for the conductance changes of depolarizing bipolars.

However, this interpretation could be complicated by simultaneous changes in the horizontal cell which in turn might affect the bipolar. If the depolarizing bipolar depended on the horizontal cell for maintaining normal light-evoked activity, the c-f effects on horizontal cells might lead to a loss of light responsiveness in the depolarizing bipolar. A further discussion of this problem involves considerable speculation but we cannot eliminate the possibility that the action of a c-f medium is principally at the horizontal cell level, and that a special interaction exists between the horizontal cell and DPB which is fundamentally different from the interaction of the horizontal cell and HPB.

Amacrine Cells The loss of the on depolarization of amacrine cells in a c-f medium has two possible explanations. The first is that the depolarization is abolished because of a loss of the input from the depolarizing bipolar. A second possibility, however, must be considered. If the amacrine cells maintained a high intracellular chloride concentration, an increase in chloride permeability would depolarize the cell. In this way the loss of the on depolarization would be due to the loss of a chloride-dependent mechanism at the amacrine cell level; persistence of the off depolarization in a c-f medium would mean that this response component is dependent on a different ionic mechanism, presumably increased sodium conductance. Here the transient effects of a c-f medium strongly argue against this possibility. If the latter mechanism were involved, then a c-f medium would initially exaggerate the on response in comparison to the off response. Our findings show that both on and off responses are equally enhanced in a c-f environment. This would argue against the view that chloride ions are critical for amacrine cell depolarization and favor the idea that the loss of the on depolarization reflects a loss of, and therefore a connection with, the DPB. An extension of this view would suggest that the light-evoked depolarization of amacrine cells is probably dependent on sodium.

Since the off depolarization of amacrine cells persists in a c-f medium, it identifies a connection with the hyperpolarizing bipolar. Thus amacrine cells receive input from both the depolarizing and hyperpolarizing bipolars. In this arrangement the loss of input from the depolarizing bipolar should unmask a hyperpolarizing component during the light stimulus; this was observed in some cells (see Fig. 6).

In summary, a c-f environment has a selective, reversible effect on the retinal network. Both depolarizing and hyperpolarizing responses are chloride sensitive and may depend on transmembrane movements of chloride ions for light-evoked electrogenesis. These findings suggest that at the outer plexiform layer, where on and off channels are first separated, the synaptic actions which result in potentials of opposite polarities probably depend on different ionic mechanisms.

Received for publication 14 March 1975.

REFERENCES

ADRIAN, R. H. 1956. The effect of internal and external potassium concentration on the membrane potential of the frog muscle. *J. Physiol. (Lond.).* **133:**631–658.

AGIN, D., and D. HOLTZMAN. 1966. Glass microelectrodes: the origin and elimination of tip potentials. *Nature (Lond.).* **211:**1194–1195.

BARKER, J. L., and R. A. NICOLL. 1973. The pharmacology and ionic dependency of amino acid responses in the frog spinal cord. *J. Physiol. (Lond.).* **288:**259–277.

BAYLOR, D. A., M. G. F. FUORTES, and P. M. O'BRYAN. 1971. Receptive fields of cones in retina of the turtle. *J. Physiol. (Lond.).* **214:**256–294.

BROWN, J. E., and L. H. PINTO. 1974. Ionic mechanism for the photoreceptor potential of the retina of *Bufo marinus. J. Physiol. (Lond.).* **236:**575–592.

CARPENTER, D. O. 1973. Electrogenic sodium pump and high specific resistance in nerve cell bodies of the squid. *Science (Wash. D.C.).* **179:**1336–1337.

CERVETTO, L., and M. PICCOLINO. 1974. Synaptic transmission between photoreceptors and horizontal cells in the turtle retina. *Science (Wash. D.C.).* **183:**417–418.

COLE, K. S., and J. W. MOORE. 1960. Liquid junction and membrane potentials of the squid giant axon. *J. Gen. Physiol.* **43:**971–980.

COOMBS, J. S., J. C. ECCLES, and P. FATT. 1955. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory postsynaptic potential. *J. Physiol. (Lond.).* **180:**326–373.

DACHEUX, R. F., and R. F. MILLER. 1976. Photoreceptor-bipolar cell transmission in the perfused retina eyecup of the mudpuppy. *Science* (*Wash., D.C.*). **191:**963–964.

DOWLING, J. E., and H. RIPPS. 1973. Effects of magnesium on horizontal cell activity in the skate retina. *Nature (Lond.).* **242:**101–103.

HERTOG, A. DEN. 1973. Some further observations on the electrogenic sodium pump in non-myelinated nerve fibers. *J. Physiol. (Lond.).* **231:**493–509.

HODGKIN, A. L., and P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol. (Lond.).* **148:**127–160.

KANEKO, A. 1970. Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. *J. Physiol. (Lond.).* **207:**623–633.

KERKUT, G. A., and R. C. THOMAS. 1964. The effect of anion injection and changes in the

external potassium and chloride concentration on the reversal potentials of the IPSP and acetylcholine. *Comp. Biochem. Physiol.* **11**:199–213.

KEYNES, R. D. 1963. Chloride in the squid axon. *J. Physiol. (Lond.).* **169**:690–705.

KLEINSCHMIDT, J. 1973. Biochemistry and Physiology of Visual Pigments. Springer-Verlag, Berlin.

KORENBROT, J. I., and R. A. CONE. 1972. Dark ionic flux and the effects of light in isolated rod outer segments. *J. Gen. Physiol.* **60**:20–45.

MILLER, R. F., and R. F. DACHEUX. 1973. Information processing in the retina: Importance of chloride ions. *Science (Wash. D.C.).* **181**:266–268.

MILLER, R. F., and R. F. DACHEUX. 1975 *a*. Chloride-sensitive receptive field mechanisms in the isolated retina-eyecup of the rabbit. *Brain Res.* **90**:329–334.

MILLER, R. F., and R. F. DACHEUX. 1976. Synaptic organization and ionic basis of on and off channels in the mudpuppy retina. II. Chloride-dependent ganglion cell mechanisms. *J. Gen. Physiol.* **67**:661–678.

MOTOKIZAWA, F., J. P. REUBEN, and H. GRUNDFEST. 1969. Ionic permeability of the inhibitory postsynaptic membrane of lobster muscle fibers. *J. Gen. Physiol.* **54**:437–461.

NELSON, R. 1973. A comparison of electrical properties of neurons in *Necturus* retina. *J. Neurophysiol.* **36**:519–535.

O'BRYAN, P. M. 1973. Properties of the depolarizing synaptic potential evoked by peripheral illumination in cones of the turtle retina. *J. Physiol. (Lond.).* **235**:207.

OOMURA, Y., H. OOYAMA, and M. SAWADA. 1965. Ionic basis of the effect of ACh on Onchidium D and H neurons. *Int. Congr. Physiol. Sci. [Proc.] 23rd.* **6**:913.

PINTO, L. H., and W. L. PAK. 1974. Light-induced changes in photoreceptor membrane resistance and potentials in gecko retinas. II. Preparations with active lateral interactions. *J. Gen. Physiol.* **64**:49–69.

SILLMAN, A. J., H. ITO, and T. TOMITA. 1969. Studies on the mass receptor potential of the isolated frog retina. II. On the basis of the ionic mechanism. *Vis. Res.* **9**:1443–1451.

TAKEUCHI, A., and N. TAKEUCHI. 1967. Anion permeability of the inhibitory postsynaptic membrane of the crayfish neuromuscular junction. *J. Physiol. (Lond.).* **191**:575–590.

TOYODA, J. 1973. Membrane resistance changes underlying the bipolar cell response in the carp retina. *Vis. Res.* **13**:283–294.

TOYODA, J., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gecko*. *Vis. Res.* **9**:435–463.

TRIFONOV. JU.A. 1968. Study of synaptic transmission between photoreceptors and horizontal cells by means of electric stimulation of the retina. *Biofizika.* **13**:809–817.

TRIFONOV, JU.A., and A. L. BYZOV. 1965. The response of the cells generating S-potential on the current passed through the eyecup of the turtle. *Biofizika.* **10**:673–680.

WALOGA, G. 1975. Ionic mechanism for the generation of S-potentials: Horizontal cells in isolated *Axolotl* retina Ph.D. Thesis. Purdue University, West Lafayette, Ind.

WEIGHT, F. F. 1974. Physiological mechanisms of synaptic modulation. The Neurosciences: Third Study Program. The Rockefeller University Press, New York. 929–941.

WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of retina of mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**:339–355.

WINKLER, B. S. 1973. Dependence of fast components of the electroretinogram of the isolated rat retina on the ionic environment. *Vis. Res.* **13**:457–463.

WOODBURY, J. W., and P. R. MILES. 1973. Anion conductance of frog muscle membranes: one channel, two kinds of pH dependence. *J. Gen. Physiol.* **62**:324–353.