

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

II. *Chloride-Dependent Ganglion Cell Mechanisms*

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ABSTRACT Extracellular ganglion cell recordings in the perfused mudpuppy eyecup show that a chloride-free (c-f) perfusate abolishes the center and surround excitation of *on-center* cells, the surround excitation of *off-center* cells, and the on discharge of *on-off* cells. These changes in ganglion cell receptive field organization are anticipated in view of the effects of a c-f environment on the neurons which are presynaptic to the ganglion cells. However, chloride-dependent inhibitory postsynaptic (IPS) responses have been observed in *on-off* ganglion cells. These inhibitory postsynaptic potentials (IPSP's) are preceded by (EPSP's) excitatory postsynaptic potentials and are apparently mediated by amacrine cells. The light-activated hyperpolarization of off cells is not the result of a chloride-dependent IPSP and probably results from disfacilitation.

INTRODUCTION

Removing chloride ions from the external environment of the retina results in selective changes in the retinal network (Miller and Dacheux, 1973; 1975 *a*; 1976 *a*). Intracellular recording experiments in the perfused mudpuppy eyecup show that the light-evoked responses of the depolarizing bipolar and horizontal cell are abolished in a chloride-free (c-f) medium. These studies demonstrated that chloride-dependent inhibitory postsynaptic potential (IPSP) mechanisms are not the predominant mode of synaptic interaction between neuronal elements lying presynaptic to the ganglion cell. In the present study we have examined the effect of a c-f medium on the ganglion cells themselves in order to evaluate possible chloride-dependent mechanisms at this level. In general, intracellular recording experiments and models of retinal connections based on anatomical findings have suggested that the main features of ganglion cell receptive field organization are determined by neural interactions before the ganglion cell layer, and that the ganglion cell summates this integrated activity (Dowling, 1968; Werblin and Dowling, 1969). The observations of this study show that while this view may explain the organization of on- and off-center cells, on-off cells have integrative properties at the level of the ganglion cell. Chloride-

dependent IPSP generation has been studied in these cells; these IPSP's are probably mediated by amacrine cells (Werblin and Copenhagen, 1974). On the other hand, the hyperpolarizing response of the off ganglion cell is not a Cl^- -dependent response and probably results from disfacilitation.

METHODS

Details of the mudpuppy perfusion technique were outlined in the previous paper (Miller and Dacheux, 1976 *a*). *Necturus maculosus* were used in all experiments. Intracellular recordings were obtained with high resistance, beveled micropipettes while the eyecup was initially perfused with normal Ringer. Extracellular recordings were obtained with glass insulated tungsten (Levick, 1972) or insl-x coated insect pins (Green, 1958). Ganglion cells were identified by the presence of impulse activity, and classification of cell type was based on the response pattern to small spot, diffuse, and annular light stimulation. Identification of center-surround organization often required a constant small spot illumination while intermittently flashing a concentrically aligned annulus. Unless otherwise indicated, the small spot stimulus was 240 μm in diameter and the annulus had an inner diameter of 600 μm and an outer diameter of 2 mm.

Intracellular ion injection was accomplished by passing current through electrodes filled with 2 M KCl or 2 M K acetate using a bridge circuit. Illustrations were photographically reproduced from the data displayed on a storage oscilloscope (Tektronix 5103, Tektronix, Inc., Beaverton, Ore.) or a penwriter (Brush 260, Gould, Inc., Cleveland, Ohio).

RESULTS

Extracellular Ganglion Cell Recording: Transient c-f Effects

Intracellular recordings of retinal neurons show that the initial effects of a c-f perfusate include a transient depolarization of many cells including some receptors (Miller and Dacheux, 1976 *a*). This transient depolarization was associated with a "silent period" during which cell responses were abolished or small in amplitude. Ganglion cell recordings show an analogous sequence of events. These transient c-f changes were similar for each class of ganglion cell. The first change consisted of a sudden, brief (10–20 s) increase in impulse activity unrelated to light stimulation, beginning 10–20 s after initiating the c-f perfusate. This was followed by a period of 10–30 s during which the cells were silent and would not respond to light stimulation. In a few cells we observed a silent period which was not preceded by an excitatory phase. After the silent period the normal light-evoked discharge pattern was restored. On- and off-center cells which responded with only an on or off discharge to a diffuse light stimulus in the control perfusate sometimes displayed both on and off discharge soon after the silent period. The additional discharge component was always of a longer latency than the center-mediated discharge. During a period which was highly variable (2–21 min), the on activity of all ganglion cells displayed an increasingly longer latency and was eventually abolished. Also the off discharge which remained in a c-f environment displayed a longer latency than in the control perfusate. Impulses were usually smaller in a c-f environment than in the control. On returning to the control environment, the ganglion cells which were

still responsive to light stimulation showed an early silent period. Recovery to a short latency response required approximately the same amount of time as that required to see a loss of the discharge during the administration of the c-f medium. In the following section the c-f-induced receptive field changes are those observed under "steady-state" c-f conditions and the transient effects have been neglected.

Extracellular Ganglion Cell Recordings: Steady-State Receptive Field Alterations

ON CELLS As the findings in Fig. 1 illustrate, on-center cells were insensitive to light stimulation under steady-state conditions in a c-f environment. The

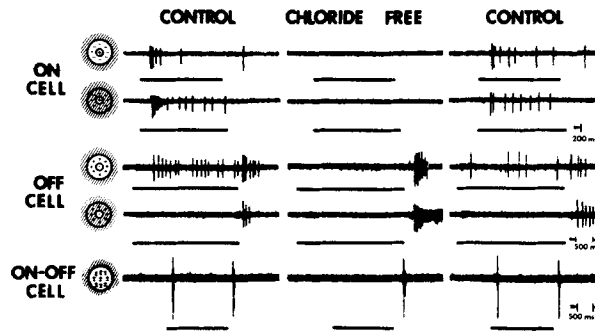


FIGURE 1. Summary of steady-state c-f effects on single-unit ganglion cells studied in perfused eyecup. Notation on left indicates cell type and diagrams on left indicate, by the absence of shading, whether diffuse or small spot stimulation was employed (i.e., responses in the upper horizontal row were evoked by diffuse light stimulation, whereas those in the second row were evoked by small spot stimulus). Left-hand column shows initial discharge pattern in normal perfusate; middle column shows steady-state alterations of activity in a c-s environment; right-hand column shows reversibility of c-f effects after returning to normal Ringer. On cell: Upper trace shows short latency (140 ms) on discharge and single long latency (275 ms) impulse at off. Lower trace shows sustained on discharge to 240- μ m light stimulus. Note that the impulses in response to small spot stimulus show progressive accommodation, followed by recovery as frequency of discharge declines. Impulse accommodation is a common observation and readily accounts for differences in impulse amplitude in all cells in this figure. Middle column shows lack of discharge to diffuse or small spot light stimulation in c-f medium. Reversibility is shown in right-hand column. Off cell: Diffuse light stimulus evoked long latency (700 ms) on discharge in control (upper trace); small spot stimulus (240 μ m) evoked off discharge (100 ms); diffuse and small spot stimulus evoked off discharge of increased latency (upper trace: 340; lower trace: 300 ms) in c-f. Recovery of normal discharge pattern shown in right-hand column. On-off cell: Diffuse light stimulus evoked on and off discharge of nearly equal latency in control (on = 140 ms; off = 125 ms). On discharge abolished in c-f environment, off discharge longer in latency compared to control (265 ms). Recovery of on discharge shown in right-hand column. Irradiance: small spot 4.6×10^{-7} W/cm²; diffuse stimulation, 3.7×10^{-7} W/cm².

loss of light-evoked activity included both center and surround excitation. The left-hand column (upper two horizontal rows) of Fig. 1 illustrates the discharge pattern of an on-center cell in response to a diffuse (upper trace) and a 240- μm (lower trace) light stimulus. The diffuse light stimulus evoked a short latency (140 ms) on discharge and a single long latency (275 ms) spike after the termination of the flash. The small spot stimulus evoked a more sustained discharge from the cell but no off discharge was apparent. Note that the response to the small spot stimulus consisted of progressively smaller spikes suggesting some accommodation during the train of impulses. Impulse accommodation was a common finding in mudpuppy recordings and probably accounts for the variations in spike amplitude throughout Fig. 1. The small spot and diffuse light stimuli demonstrate the center-surround organization of this cell. After 15 min in the c-f environment (middle column), the cell was unresponsive to both diffuse and small spot light stimulation, regardless of stimulus intensity. The right-hand column shows the reversibility of this effect observed 11 min after returning to the control perfusate. Two of the 10 cells showed evidence of surround excitation in response to a diffuse light stimulus. Six cells showed only an on discharge to the diffuse light stimulus, but an off discharge could be elicited by flashing the annulus using steady small spot illumination of the center. Thus, 8 of the 10 cells were considered to be antagonistically organized "on-center" cells. Two cells did not have an off discharge to either the diffuse stimulus or the center adaptation plus annulus technique, and therefore are considered to be "on" cells; the discharge of these cells was also abolished in a c-f medium.

OFF CELLS All off responding cells were "off-center" cells in that an antagonistic on discharge could be evoked under appropriate stimulus conditions; each cell remained responsive to light off in a c-f medium, but the surround excitation was abolished by this procedure. Fig. 1 (third and fourth horizontal rows) illustrates an off cell which responded with a long latency (700 ms) sustained discharge at the onset of a diffuse light stimulus and a short latency off discharge (100 ms) at light off. After 6 min in a c-f environment, the cell responded only at light off to both the diffuse and small spot stimulus. The latency of the off discharge was more than tripled in the c-f medium (340 ms, upper trace; 300 ms, lower trace). The right-hand column shows the reversibility of this effect observed 4 min after returning to the control Ringer. The off-center cell of Fig. 1 was exceptional because in most off-center cells, surround excitation required flashing an annulus while adapting the center with steady small spot illumination. In all cases the on discharge evoked by this method had a longer latency than the off discharge.

In addition to the increased latency of the off discharge, changes in "summation time" were observed in a c-f environment. For example, if a 0.5-s stimulus evoked an off discharge in the control Ringer, it was sometimes necessary to use a 1-s stimulus to evoke an off discharge in the c-f medium. Also, a light stimulus which would evoke an off discharge on one occasion would sometimes not evoke an off discharge when presented 5 s later. The latter effect seemed to result

from periodic fluctuations in the excitability of the cell, probably related to the c-f-induced spontaneous ganglion cell activity described below.

ON-OFF CELLS The on discharge of on-off cells was lost in a c-f environment whereas the off discharge remained. The lowest horizontal row of Fig. 1 shows the response of an on-off cell to a diffuse light stimulus. On-off cells were characterized by on (140 ms, Fig. 1, lowest trace, left-hand response) and off (125 ms) activity of nearly equal latency to both small and large spot light stimulation. After 7 min in a c-f environment, the on discharge was absent but the off discharge remained though an increased latency was observed (265 ms). Six of the on-off cells were tested for motion selectivity with a forward-backward moving slit rotated through 360°, but none showed motion-selective properties.

In summary, light-sensitive ganglion cell activity in a c-f medium under steady-state conditions is restricted to the center-mediated discharge of off cells and the off discharge component of on-off cells. The off responses were of longer latency than the latency values observed in the control environment.

SPONTANEOUS ACTIVITY Most ganglion cells were not spontaneously active in the control Ringer, but developed some degree of spontaneous (dark) activity in the c-f environment. Spontaneous activity was observed in off and on-off cells; the on cells were silent in a c-f environment, though these cells sometimes developed spontaneous activity before termination of light-evoked discharge. The spontaneous activity had several different patterns. One pattern consisted of bursts of impulses which reoccurred every 1-3 s; each burst consisted of one to five impulses. Another type of spontaneous activity consisted of a periodic increase in discharge lasting a few seconds, followed by a period during which no spontaneous activity was evident. A third phenomenon included spreading depression. The termination of long-duration light stimuli (10 s or more) was associated with a high frequency burst of impulse activity; during this burst, successive impulses were progressively smaller until discharge ceased. This was followed by a period of 30 s to 4 min during which ganglion cells did not respond to light stimulation. Recovery of light-evoked and spontaneous impulse activity began with small amplitude impulses, followed by progressively larger spikes. Intracellular recordings from Müller cells, ganglion cells, amacrine cells, and hyperpolarizing bipolars have shown that all of these cells are depolarized during spreading depression episodes; the depolarization of Müller cells is especially large. These observations will be presented in more detail in a future communication: a "c-f retina" offers a promising experimental preparation for further studies of spreading depression.

INTRACELLULAR GANGLION CELL RECORDINGS Intracellularly recorded responses of three different ganglion cell types are illustrated in Fig. 2. Recordings of the upper row were obtained from an on-off cell in response to a diffuse light stimulus (left trace), followed 5 s later by an annulus, followed again by a diffuse light stimulus (right-hand recording). Diffuse stimulation evoked on and off impulse activity followed by an on and off transient hyperpolarization (arrows). The annulus flash evoked a small excitatory postsynaptic potential (EPSP) at off

but the transient hyperpolarizing on-off responses are the prominent feature of this recording. This observation suggests that the hyperpolarizing mechanism has a larger receptive field than the depolarizing excitatory mechanism. Responses recorded from an on-center (lower row, left trace) and an off-center (right trace) cell were elicited by diffuse light stimuli which did not evoke

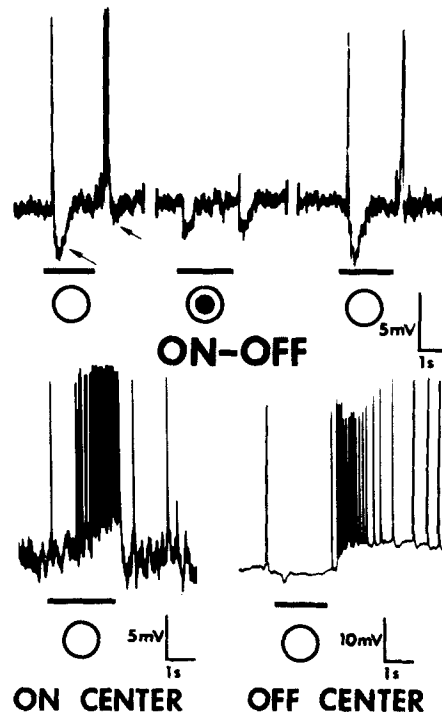


FIGURE 2. Intracellular recordings from three different ganglion cell types. Upper traces show response of on-off ganglion cell to diffuse light stimulus (left recording), followed by annulus (middle response), followed by second diffuse light stimulus (right trace). Diffuse light stimulus evoked on and off impulse activity followed by on and off hyperpolarizing transient responses (arrows). Annulus evoked on and off hyperpolarizations with small off EPSP, but no impulse activity. Lower traces show on-center and off-center cells in response to diffuse light stimulus which did not evoke surround excitation. Note that on and off hyperpolarizations are not observed in on- and off-center cells. All traces are photographic reproductions of responses recorded on penwriter which caused some attenuation of impulse activity. Stimulus irradiance equal for all stimuli: 3.7×10^{-7} W/cm².

antagonistic surround excitation. On and off hyperpolarizations are not evident in on- and off-center cells. The hyperpolarizing responses of on-off cells have the appearance of IPSP's and we have examined the possibility that these responses, like the IPSP's of other neurons (Coombs et al., 1955) are dependent on chloride.

In the mudpuppy, both on-off ganglion cells and amacrine cells respond similarly: Light stimulation results in on and off PSP's and both cell types

generate impulse activity (Werblin and Dowling, 1969). A question is thus presented as to how one differentiates on-off ganglion cell recordings from amacrine cell recordings. We have studied this problem extensively, using antidromic optic nerve stimulation to assist in cell identification. Some of these

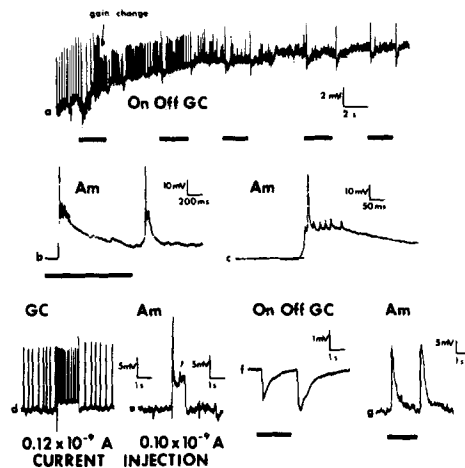


FIGURE 3. Trace *a* shows on-off ganglion cell recording obtained immediately after cell penetration. Injury depolarization is associated with constant impulse activity. Large negative deflection after onset of first light stimulus (dark bar) due to readjustment of amplifier offset. A one-half gain change introduced to permit display of response without further offset adjustment. As impulse activity becomes smaller, light stimuli evokes on and off EPSP (a small spike component may be associated with the EPSP response) IPSP sequence. Fig. 3 *b* shows intracellular amacrine cell response to a diffuse light stimulus. Large and small amplitude spikes are associated with a large amplitude on and off EPS response. Fig. 3 *c* shows amacrine cell recording at fast time base to facilitate resolution of amacrine cell response components. Initial amacrine cell response consists of slow rising EPSP followed by fast prepotential (FPP). Notch of FPP is followed by large amplitude spike and several smaller spikes. (*d*) Intracellular ganglion cell recording. Spontaneous activity due to slight injury depolarization. Depolarizing current injection gives relatively sustained level of impulse activity. (*e*) Depolarizing current injection in amacrine cells evokes single large amplitude spike; small amplitude spike is also apparent (arrow). (*f*) Depolarized on-off ganglion cell shows on-off hyperpolarizing response. (*g*) Depolarized amacrine cell shows on-off depolarizing responses. Traces *a*, *d*, *e*, *f*, and *g* are photographic reproductions of penwriter recordings. Traces *b* and *c* were photographed from storage screen oscilloscope. Diffuse light stimuli used throughout. Irradiance: 3.7×10^{-7} W/cm².

data have been published (Miller and Dacheux, 1976 *c*). In the vast majority of recordings the distinction between the two cell types is not difficult and identification can be made on the basis of impulse activity or PS response pattern.

Fig. 3 illustrates the differentiating features observed in on-off ganglion cells and amacrine cells. It is a common experience in recording from both cell types that the responses observed just after cell penetration are associated with im-

pulse activity. However, these units often depolarize rapidly and the impulse mechanism is inactivated leaving PS responses. Trace *a* of Fig. 3 shows a depolarizing on-off ganglion recorded immediately after penetration. During the initial period of penetration-induced injury depolarization, a relatively constant level of impulse activity is evident, and light stimuli elicit early excitation, followed by hyperpolarizing responses at light on and off. The large negative deflection observed after the onset of the first light stimulus represents a readjustment of the offset control. A one-half gain change was introduced to permit recording of the entire sequence without further adjustment. At the time the recording reached a relatively steady level, the response consisted of on and off EPSP responses (plus perhaps a small spike) followed by transient on and off hyperpolarizing responses; the hyperpolarizing responses are larger during the later segments of the recording. These on-off cells are easily activated by antidromic optic nerve stimulation.

Amacrine cells show two different types of impulse activity (Miller and Dachaux, 1976 *c*). Trace *b* of Fig. 3 shows an amacrine cell response evoked by a diffuse light stimulus. A large on and off EPSP is associated with both large and small amplitude spikes. Trace *c*, displayed at a faster time scale, shows the large and small amplitude spikes more clearly. The earliest amacrine cell response consists of a slow rising EPSP, followed by a fast prepotential (FPP). A large spike arises just after the peak of the FPP, and is followed by several small spikes. Most often, the large spike arises directly from the peak of the FPP. FPP's have previously been described (Spencer and Kandel, 1961); they are considered to be spikes of dendritic origin, low in amplitude at the level of the soma because of electrotonic decay between the site of generation and the cell body. Our analysis suggests that the small spikes and FPP are identical and are generated by the amacrine dendrites, whereas the large spikes are probably of somatic origin. Thus, amacrine cells generate two different types of impulses but only a single class of impulses are observed in ganglion cell recordings.

A second difference between amacrine cell somatic spikes and ganglion cell spikes is that the latter, especially those of 50 mV or more, show an initial segment-soma dendritic (IS-SD) break (Eccles, 1957). However, somatic spikes of amacrine cells with or without FPP's do not show evidence of an IS-SD break, consistent with the axonless morphology of these cells. A further difference between amacrine cells and ganglion cells relates to accommodation. Intracellular depolarizing current injection of ganglion cells (trace *d*) results in a relatively sustained level of impulse activity (an early phase of high frequency activity is apparent). This is true for on, off, and on-off cells. It is possible that some differences in accommodation exist between these ganglion cell types, but in all ganglion cell recordings, it is possible to evoke sustained impulse activity within a range of depolarizing current injection. On the other hand, current injection in amacrine cells (trace *e*) gives rise to a single somatic spike (a dendritic spike was also evoked at arrow), consistent with the idea that the somatic spike is rapidly accommodated, and in this respect resembles the accommodation level described in squid axon (Hodgkin, 1964). This difference readily accounts for our observation that injury depolarization of ganglion cells is initially accompanied

by a constant impulse discharge, whereas injury depolarization of amacrine cells is not. This distinction does not apply to the dendritic spikes of amacrine cells which do not rapidly accommodate. Once ganglion cells and amacrine cells depolarize to some level, the on-off ganglion cell consists of an EPSP-IPSP sequence, whereas the amacrine cell consists of EPSP's (Fig. 3 *g*). Further depolarization of on-off ganglion cells often obscures the initial EPSP leaving an on and off IPSP (Fig. 3 *f*). In fact this seems to be the commonly encountered state of an on-off ganglion cell impaled by a micropipette in the perfused eyecup preparation. Thus in a comparable state of depolarization, in which impulse-generating mechanisms are inactivated, the amacrine cell response consists of on and off EPSP's with no obvious IPSP components. An additional difference between the two cell types relates to the EPSP amplitude. Amacrine cells generate "giant" EPSP's with a range of 4-30 mV; the EPSP's of on-off ganglion cells rarely exceed 5 mV.

Fig. 4 *a* shows the EPSP-IPSP sequence of an on-off ganglion cell in the absence of impulse activity. Fig. 4 *b* shows the on and off IPSP observed after further depolarization has obscured the EPSP. In this response, the change in bridge balance was monitored while passing an intermittent + current through the recording electrode. An increased negative deflection shows that the IPSP's were associated with an increased conductance. Near the end of the trace a persistence in the bridge imbalance may indicate a prolonged conductance change, or a permanent change in electrode resistance; the latter problem is commonly encountered with high resistance electrodes.

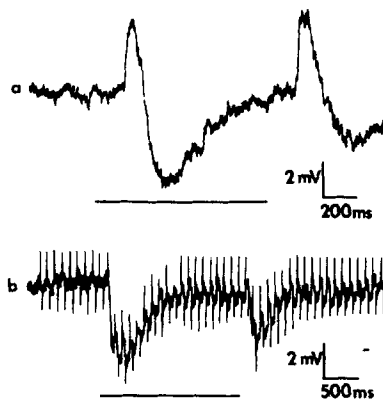


FIGURE 4. Trace *a* shows intracellular recording from on-off ganglion cell after injury depolarization inactivated the impulse-generating mechanism. On and off EPSP's are followed by transient on and off IPSP's. On and off EPSP responses nearly equal in latency (on = 200 ms; off = 186 ms). Trace *b* shows change in bridge balance during IPSP. Electrode positive current (0.5×10^{-9} A) passed intermittently; appearance of downward deflection during IPSP indicates conductance increase at on and off. Persistent bridge imbalance at end of trace may represent prolonged conductance change or change in electrode resistance. Diffuse light stimulation for both traces. Irradiance: 4.6×10^{-7} W/cm².

In cat spinal cord, Coombs et al. (1955) used intracellular ion injections to show that IPSP's in motoneurons result from an increased chloride conductance. Fig. 5 shows on and off IPSP's inverted to depolarizing responses during a current injection of 1×10^{-9} A (electrode negative) passed through a KCl-filled electrode. This current injection was maintained for 30 s, after which the response remained permanently inverted for more than 100 s, at which time the recording was lost. Chloride injection studies were carried out in 17 cells using -1×10^{-9} A for 30 s for each cell. Eleven recordings were lost between 22 and 300 s after the injection; each recording showed positive on and off responses during this period. Six recordings showed recovery to hyperpolarizing responses between 45 and 110 s after current injection. A much different result was obtained if the injections were carried out using K acetate-filled micropipettes. Fig. 6 shows a decreased on-off IPSP amplitude during a current injection of -0.5×10^{-9} A (second trace) and 0.75×10^{-9} A (third trace) passed through an electrode filled with 2 M K acetate. The current was increased to 1.0×10^{-9} A (fourth trace) which inverted the response to a depolarization; this level of current remained on for 30 s after which IPSP recovery was observed. At 5 s the IPSP amplitude was about 0.94 control amplitude, and at 10 s the IPSP was larger than the control response. Eleven cells were studied with K acetate injection. Nine cells recovered to preinjection amplitudes within 10 s and four of these cells showed a recovery to greater than control amplitude. Two cells showed rapid recovery to IPSP amplitudes which were smaller than control values and both recordings quickly deteriorated. Responses which were larger than preinjection controls could result from further enhancement by injury depolarization or a reduction in intracellular chloride from the negative current injection (Coombs et al., 1955).

The behavior of IPSP's to intracellular chloride injections suggests that chloride plays a role in IPSP generation. Unequivocal evidence for this has been obtained from the behavior of IPSP's during perfusion with a c-f medium. Intracellular recording experiments and ganglion cell studies show a c-f environment results in a loss of on ganglion cell activity because of selective changes within the retinal network (Miller and Dacheux, 1973, 1975 a). In the mud-puppy, the loss of the on discharge required prolonged exposure (3-21 min) to a c-f environment; during the early period of a c-f perfusate, a transient silent period was often seen, after which the retinal network as indicated by ganglion cell discharge was essentially normal. It was during the early c-f period that the recordings illustrated in Fig. 7 were obtained. The left-hand section of Fig. 7 shows an on-off IPSP evoked by a diffuse light stimulus in a normal Ringer medium. The middle section was recorded 30 s after introducing a c-f perfusate and shows reversal of on and off IPSP's accompanied by a slight (less than 2 mV) depolarization of the ganglion cell. The inverted IPSP's continue to increase in amplitude during the c-f exposure. Forty seconds after returning to the normal Ringer, the responses were reversed to hyperpolarizations accompanied by a hyperpolarization of the ganglion cell. The initial on-off responses of the right-hand section show biphasic IPSP's which begin with a depolarization followed by a hyperpolarization. This observation suggests that the environment surround-

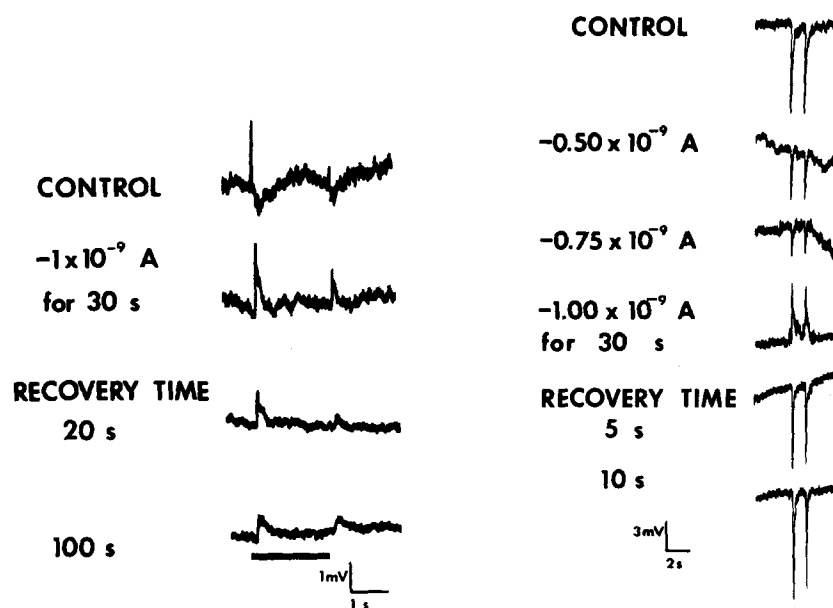


FIGURE 5

FIGURE 6

FIGURE 5. Intracellular chloride injection study of hyperpolarizing response of on-off ganglion cells. Control recording shows on and off EPSP responses followed by on and off hyperpolarizations. A partial spike is probably associated with the on EPSP. Current injection of 1×10^{-9} A (electrode negative) through KCl electrode inverted hyperpolarizing response to depolarization during period of current injection. Current injection maintained for 30 s. Lower responses show a permanent inversion of hyperpolarizing responses for 100 s after cessation of current. Photographic reproductions of penwriter recordings. Diffuse irradiance: 3.7×10^{-7} W/cm².

FIGURE 6. Intracellular acetate injection into on-off ganglion cell. 0.5×10^{-9} A (electrode negative) applied to recording electrode filled with 2 M K acetate after balancing bridge. Note reduced IPSP amplitude compared to control. Increase in current to 0.75×10^{-9} A caused further reduction in IPSP responses. 1.0×10^{-9} A inverted responses to depolarizations. Latter current level maintained for 30 s after which recovery followed by diffuse light stimuli at a rate of $1/5$ s. At 5 s after current off, IPSP recovered to 0.94 of control; at 10 s IPSP was increased by 1.20 over control. Increased IPSP could be due to additional injury depolarization or secondary to reduced intracellular chloride concentration caused from negative current injection. Diffuse irradiance: 4.6×10^{-7} W/cm².

ing some of the inhibitory synapses experienced the increased external chloride before others. Since the biphasic response pattern was observed in both on and off responses, it suggests that on and off IPSP's may share a similar morphological distribution, and conceivably both on and off IPSP's are mediated through a common cell. The reversal of the IPSP's in a c-f environment is explained by a rapid decrease in external chloride such that an increase in chloride permeability at the ganglion cell level caused a net outward movement of chloride ions

(Kerkut and Thomas, 1964; Motokizawa et al., 1969). A return to the control environment reestablished the original chloride gradient such that an increased chloride permeability resulted in a net inward movement of chloride ions. The smaller amplitude IPSP's observed after returning to the control Ringer may be the result of a hyperpolarization (approximately 2 mV) of the ganglion cell membrane potential. Thirty-eight IPSP's were examined in this way: 36 inverted to depolarization responses and 2 disappeared as the cell was markedly hyperpolarized.

The study illustrated in Fig. 7 shows the IPSP's during a brief exposure to the c-f medium. Although chloride would tend to leak out of the cell under c-f conditions, the c-f exposure was too brief to result in a significant loss of intracellular chloride ions. We have maintained intracellular recordings from four IPSP units for more than 20 min and have observed the following sequence

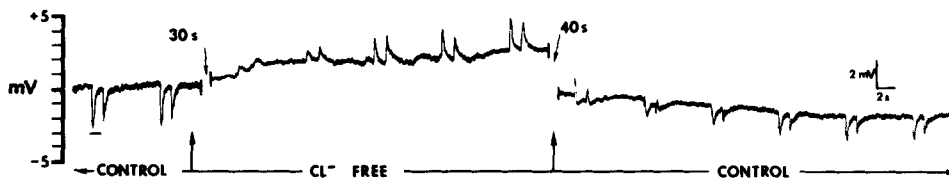


FIGURE 7. Effects of a brief exposure to c-f medium on IPSP responses of on-off ganglion cell. Left-hand section shows responses to diffuse light stimulation in control Ringer. Middle section shows reversal of IPSP's to depolarizing responses about 30 s after initiating a c-f medium. Note that the membrane potential had been depolarized by about 3 mV. Right-hand section shows repolarization of ganglion cell and reinversion of responses to hyperpolarizations. Initial two responses in right-hand section show transition with initial depolarizing responses followed by hyperpolarizing transients. This observation suggests that some synaptic regions experienced an increase in external chloride before others. Equal waveforms of on and off responses in transition records suggest on and off IPSP's are generated at similar locations and possibly by same cellular input. Diffuse irradiance: 4.6×10^{-7} W/cm².

of events. The first change like that illustrated in Fig. 7 is an inversion of the IPSP. This is followed a few minutes later (3 to 6 min) with a loss of the on response which we have attributed to a loss of the input at light on. Further exposure to a c-f environment, however, results in a gradual loss of the off IPSP. This may have been due to a loss of chloride from the cell since the probable input for this response (off response of amacrine cells) is not abolished in a c-f perfusate (Miller and Dacheux, 1976 *a*). This observation suggests that ions other than chloride may make only a small contribution to the IPSP (Takeuchi and Takeuchi, 1967).

The response characteristics of the on-off IPSP favor the view that this mechanism is mediated by amacrine cells. On-off amacrine cells and the on-off IPSP's have on latencies which are nearly equal to the off latencies and generate on-off responses to small spot, diffuse, or annular light stimulation, provided the inner diameter is of proper adjustment. Neither the amacrine cells nor the

IPSP's show evidence of antagonistic center-surround organization. Fig. 8 shows an IPSP inverted to a depolarizing response in the c-f environment compared to an intracellularly recorded amacrine cell (no impulse activity apparent); both responses show impressively similar waveforms. Also we will show in a future communication that the IPSP's of on-off ganglion cells often shows fast and slow components; the fast components may reflect synaptically mediated dendritic spike activity of the amacrine cell.

A second type of light-activated hyperpolarization is that associated with off

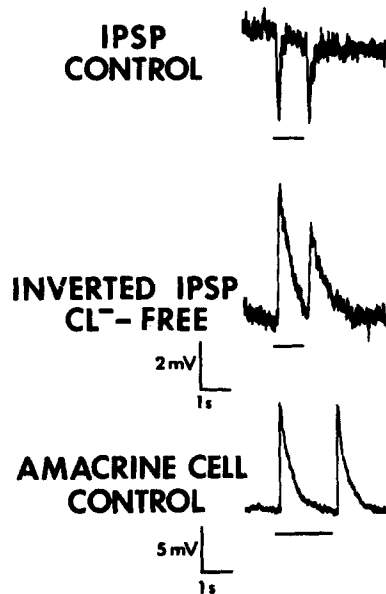


FIGURE 8. Comparison of inverted IPSP response in early c-f environment with intracellularly recorded amacrine cell response. Upper trace shows IPSP of on-off ganglion cell in control Ringer. Middle trace shows inverted IPSP 30 s after initiating c-f medium. Lower trace shows intracellular recording from cell for comparison. Diffuse irradiance: 4.6×10^{-7} W/cm².

ganglion cells. These cells are distinguished from on-off cells, because only off discharge is usually observed in response to diffuse light stimulation but also there is a sustained hyperpolarizing component to the response. Without impulse activity these cells look similar to hyperpolarizing bipolars. Thus to be identified as an off ganglion cell we required the presence of impulse activity; however, this impulse activity was often temporary and rapidly inactivated before permanent penwriter recordings were obtained. Nine off ganglion cells have been studied and each cell was similar to that shown in Fig. 9. Fig. 9, left-hand section, shows an off ganglion cell in response to a diffuse light stimulus. The traces to the right were obtained from a different off cell after impulse activity was inactivated from injury depolarization. Within 10 s after initiating a c-f environment, the ganglion cell was initially hyperpolarized and then slowly

depolarized to a net depolarization of about 1 mV. The depolarization is not shown but the trace is displaced according to the observed change in potential. Returning to the control environment resulted in a slight depolarization and a decreased response amplitude, probably due to further injury depolarization. In contrast to the IPSP mechanism of on-off ganglion cells, the hyperpolarization of off cells is not due to Cl^- -dependent IPSP since reversal to a depolarizing response did not occur. This observation suggests that another mechanism must be responsible for the hyperpolarizing responses of off ganglion cells. One possibility is that a transmitter released during light stimulation results in an increased permeability to K^+ . This mechanism has been demonstrated for hyperpolarizing responses of the snail (Gerschenfeld and Chiarandini, 1965) and *Onchidium* (Oomura et al., 1965). It also appears to be the mechanism of Ach inhibition in the heart (Castillo and Katz, 1955; Trautwein et al., 1956). In the

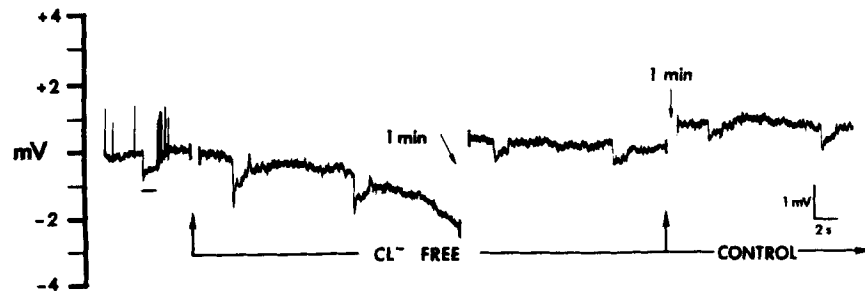


FIGURE 9. Intracellularly recorded response of off-center ganglion cell. Response on left shows light-evoked hyperpolarization followed by impulse activity at light off. Remainder of trace recorded from a second off cell after impulse mechanism was inactivated by injury depolarization. C-f medium caused initial hyperpolarization followed by depolarization of cell. (Depolarization phase not illustrated.) No reversal of light-evoked hyperpolarization occurred during exposure to c-f medium, though response was reduced in amplitude. Return to normal Ringer resulted in further depolarization with no change in light-evoked response amplitude. Diffuse irradiance: 4.6×10^{-7} W/cm².

previous study (Miller and Dacheux, 1976 *a*) we showed that the off cells must be subserved by the hyperpolarizing bipolar. Also the application of synaptic blocking agents leads to a hyperpolarization of hyperpolarizing bipolars (Dacheux and Miller, 1976) suggesting that a dark-released receptor transmitter has a depolarizing action on this cell. Thus a second possibility is that a depolarizing transmitter is released by the bipolar in the dark and that light stimulation causes a disfacilitation. We do not have strong evidence to decide between these two possibilities. However, it is commonly observed that injury depolarization progressively decreases the response amplitude of the off cell hyperpolarization. If the K^+ mechanism is the basis of this hyperpolarization, one might expect to see an increased hyperpolarization for the same reason that a depolarization increases the amplitude of IPSP's in on-off cells. However, a decrease in the hyperpolarizing response, associated with ganglion cell depolarization, is consistent with the disfacilitatory mechanism since the soma-dendritic tree would be

depolarized to a level closer to the dark-mediated EPSP mechanism. This argument favors the disfacilitatory mechanism, but it is not conclusively established.

DISCUSSION

The absence of any on ganglion cell activity in a c-f environment reflects selective reversible changes in receptive field properties of the on, off, and on-off cells. The on cells became insensitive to light stimulation; surround excitation of off-center cells was abolished and the on component of the on-off cells was chloride sensitive. These findings are identical to those described in the rabbit retina (Miller and Dacheux, 1973; Miller and Dacheux, 1975 *a*), and thus two different vertebrate retinas show a fundamental similarity in their sensitivity to the removal of chloride ions. It is possible that the removal of chloride may reveal a type of organization which is common to all vertebrate retinas.

In addition to the alterations in ganglion cell receptive field properties, a c-f environment produced changes in spontaneous activity of ganglion cells. The spontaneous discharge appears to reflect fluctuations in the excitatory input to the off and on-off ganglion cells, since spontaneous activity was not evident in cells which were unresponsive to light stimulation (on cells). Similar changes were observed in ganglion cell activity in the c-f rabbit retina (Miller and Dacheux, 1975 *a*).

Additional factors which might contribute to more "excitable" ganglion cells are: (a) A removal of chloride could increase the membrane resistance of ganglion cells and other neurons such that excitatory activity would be less attenuated electrotonically and therefore more influential in regulating ganglion cell activity (Takeuchi and Takeuchi, 1967). An increase in membrane resistance would also increase the time constant of the membrane and explain the increase in discharge latency observed in off and on-off cells. The early c-f-induced depolarization observed in ganglion cells is consistent with a significant chloride permeability of these cells. However, as was shown in the preceding paper, other cells including some receptors are also initially depolarized by this procedure, and it is therefore difficult to distinguish between direct effects on ganglion cells vs. synaptically mediated changes. (b) A loss of chloride-dependent IPSP's at the ganglion cell level would make the ganglion cell more excitable, since excitation would be unopposed by inhibition; the results of this study show that IPSP responses are seen in on-off ganglion cells and if the loss of IPSP's were a significant factor, one would expect spontaneous activity to be greater in on-off cells than off cells, and this was not observed. (c) A relatively depolarized state of the ganglion cell in a c-f medium could render the cell more excitable, assuming that threshold for impulse generation was less affected.

Intracellular recordings from retinal ganglion cells show that a c-f environment has a direct effect on the on-off ganglion cells themselves. In these cells we have demonstrated an on and off chloride-dependent IPSP which follows a preceding on-off EPSP response. Anatomical studies (Dowling, 1968; Dowling and Werblin, 1969) have raised the possibility that some amacrine cells may be involved in the excitatory pathway from bipolar cells to ganglion cells. Werblin

and Dowling (1969) have suggested that the on-off amacrine cells are excitatory to on-off ganglion cells because of similarities in waveform. More recently, Werblin and Copenhagen (1974) have demonstrated a hyperpolarization of the on-off ganglion cells by employing a special stimulation technique which activated amacrine cells. This amacrine-cell-mediated hyperpolarization could be due to a reduced excitatory input (disfacilitation) or a direct inhibitory influence on ganglion cells. The on-off IPSP's we have studied have strong similarities to amacrine cell responses. The results presented here provide unequivocal evidence that the IPSP is chloride dependent and that the internal chloride is normally maintained in low concentration in the cell. Thus, what appears to be an amacrine-mediated hyperpolarization is a true inhibitory effect and not a disfacilitatory influence. If the amacrine cells are inhibitory to the on-off ganglion cells, what is the pathway for excitation? In the following paper we present a model of ganglion cell receptive field pathways based on our studies of c-f effects. We propose that the on-off ganglion cells receive excitatory input from the depolarizing and hyperpolarizing bipolars, and that the amacrine cells provide the chloride-dependent IPSP pathway. According to this model ganglion cell excitation and amacrine cell responses should have nearly identical latencies, and this is demonstrated in some experimental tests of this synaptic arrangement (Miller and Dacheux, 1976 *b*). Thus, the delay between the EPSP and IPSP in on-off ganglion cells is consistent with the additional neuron interposed in the inhibitory pathway.

Intracellular recordings from off cells show that the light-activated hyperpolarization of these cells is neither inverted nor abolished in a c-f medium and is not, therefore, due to an increased chloride conductance. It seems likely that the hyperpolarization of off cells results from a light-activated decrease of a dark-mediated EPSP mechanism (i.e., disfacilitation). The possibility of dark-mediated excitatory influences is not surprising in view of recent findings which suggest that receptors release a transmitter in the dark (Trifonow and Byzov, 1965; Dowling and Ripps, 1973). Furthermore, ganglion cell recordings in the dark-adapted cat show that off-center ganglion cells have a higher level of spontaneous activity than on-center cells (Jung, 1964; Barlow and Levick, 1969) suggesting that they are in a relatively more depolarized state. It is not clear however why ganglion cells in the amphibian retina tend to show very little spontaneous activity.

The presence of Cl^- -dependent, amacrine-mediated IPSP's in the on-off ganglion cells raises interesting questions related to the possible synaptic transmitter. In a number of central nervous system (CNS) pathways (Krnjevic, 1970), Gamma amino butyric acid (GABA) has been shown to be an inhibitory transmitter whose mechanism of action is an increased chloride conductance for both post- and presynaptic inhibition (Nishi et al., 1974). Furthermore, a number of studies are consistent with the idea that GABA may be a transmitter agent for some amacrine cells (Ehinger, 1970; Ehinger and Folck, 1971; Graham, 1972) and GABA efflux from the retina has also been observed (Voaden and Starr, 1971). GABA has an inhibitory effect on ganglion cells (Noell and Lasansky, 1959). This raises the possibility that the chloride-dependent IPSP's of on-off

ganglion cells might be activated by GABA. It will be of interest for future studies to examine the effects of GABA antagonists on the IPSP's of on-off ganglion cells.

Note Added in Proof Since submitting this paper we have had an opportunity to study the hyperpolarizing response of off ganglion cells using an intermittent current injection to evaluate the resistance changes associated with this response. These observations clearly show that the light-evoked hyperpolarizing response is associated with an increased resistance and thus strongly support the idea that off ganglion cell hyperpolarization is the result of disfacilitation.

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REFERENCES

- BARLOW, H. B., and W. R. LEVICK. 1969. Changes in the maintained discharge with adaptation level in the cat retina. *J. Physiol. (Lond.)*. **212**:699-718.
- CASTILLO, J. DEL, and B. KATZ. 1955. Production of membrane potential changes in the frog's heart by inhibitory nerve impulses. *Nature (Lond.)*. **175**:1035-1036.
- 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 post-synaptic 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. 1968. Synaptic organization of the frog retina: an electron microscopic analysis comparing the retinas of frogs and primates. *Proc. R. Soc. Lond. B Biol. Sci.* **170**:205-228.
- DOWLING, J. E., and H. RIPPS. 1973. Effects of magnesium on horizontal cell activity in the skate retina. *Nature (Lond.)*. **242**:101-103.
- DOWLING, J. E., and F. S. WERBLIN. 1969. Organization of retina of the mudpuppy, *Necturus maculosus*. I. Synaptic structure. *J. Neurophysiol.* **32**:315-338.
- ECCLES, J. C. 1957. *The Physiology of Nerve Cells*. Johns Hopkins University Press, Baltimore, Md.
- EHINGER, B. 1970. Autoradiographic identification of rabbit retinal neurons that take up GABA. *Experientia (Basel)*. **26**:1063.
- EHINGER, B., and B. FOLCK. 1971. Autoradiography of some suspected neurotransmitter substances: GABA, glycine, glutamic acid, histamine, dopamine, and L-dopa. *Brain Res.* **33**:157-172.
- GERSCHENFELD, H. M., and D. J. CHIARANDINI. 1965. Ionic mechanism associated with non-cholinergic synaptic inhibition in molluscan neurons. *J. Neurophysiol.* **28**:710-723.
- GRAHAM, L. T. 1972. Intraretinal distribution of GABA content and GAD activity. *Brain Res.* **36**:476-479.
- GREEN, J. D. 1958. A simple microelectrode for recording from central nervous system. *Nature (Lond.)*. **182**:962.
- HODGKIN, A. L. 1964. *The conduction of the nervous impulse*. Sherrington Lecture VII. Charles C. Thomas, Publisher, Springfield, Ill.
- JUNG, R. 1964. Neuronale grundloggen des itell-punkelschens und der forbwahrnehmung. *Ber. Deut. Ophthal. Ges.* **66**:69-111.
- 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.
- KRNJEVIC, K. 1970. Glutamate and gamma amino butyric acid in brain. *Nature (Lond.)*. **228**:119-124.
- LEVICK, W. R. 1972. Another tungsten microelectrode. *Med. Biol. Eng.* **10**:510-515.
- 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. 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 *a*. Synaptic organization and ionic basis of on and off channels in the mudpuppy retina. I. Intracellular analysis of chloride-sensitive electrogenic properties of receptors, horizontal cells, bipolar cells, and amacrine cells. *J. Gen. Physiol.* **67**:639-659.
- MILLER, R. F., and R. F. DACHEUX. 1976 *b*. Synaptic organization and ionic basis of on and off channels in the mudpuppy retina. III. A model of ganglion cell receptive field organization based on chloride-free experiments. *J. Gen. Physiol.* **67**:679-690.
- MILLER, R. F., and R. F. DACHEUX. 1976 *c*. Dendritic and somatic spikes in mudpuppy amacrine cells: identification and TTX sensitivity. *Brain Res.* **104**:157-162.
- 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.
- NISHI, S., S. MINOTA, and A. G. KARZMAR. 1974. Primary afferent neurons: the ionic mechanism of GABA mediated depolarization. *Neuropharmacology*. **13**:215-217.
- NOELL, W. K., and A. LASANSKY. 1959. Effects of electrophoretically applied drugs and electrical currents on the ganglion cells of the retina. *Fed. Proc.* **18**:115.
- OOMURA, Y., H. OYAMA, 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.
- SPENCER, W. A., and E. R. KANDEL. 1961. Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *J. Neurophysiol.* **32**:272-285.
- TAKEUCHI, A., and N. TAKEUCHI. 1967. Anion permeability of the inhibitory postsynaptic membrane of the crayfish neuromuscular junction. *J. Physiol. (Lond.)*. **191**:575-590.
- TRAUTWEIN, W., S. W. KUFFLER, and C. EDWARDS. 1956. Changes in membrane characteristics of heart muscle during inhibition. *J. Gen. Physiol.* **40**:135-145.
- 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.
- VOADEN, M. J., and M. S. STARR. 1971. The efflux of radioactive GABA from rat retina *in vitro*. *Vis. Res.* **12**:559-566.
- WERBLIN, F. S., and D. R. COPENHAGEN. 1974. Control of retinal sensitivity. III. Lateral interactions at the inner plexiform layer. *J. Gen. Physiol.* **63**:88-110.
- WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of retina of mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**:339-355.