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SUSTAINED SYNAPTIC INPUT TO GANGLION CELLS OF MUDPUPPY RETINA

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SUMMARY

1. Intracellular responses were recorded from on-centre and off-centre ganglion cells in isolated eyecups of the mudpuppy, *Necturus maculosus*.

2. Current–voltage relations were measured in darkness, during illumination of the receptive field centre, and after chemically mediated synaptic inputs were blocked by 4 mM-cobalt chloride.

3. In on-centre cells the membrane potential in darkness was -56 ± 6 mV (mean $\pm$ S.D.). Addition of Co^{2+} resulted in an average depolarization of 10 mV and an average decrease in conductance of 2·1 nS. These results suggest that in darkness on-centre cells are tonically inhibited by synaptic input which increases conductance and has a reversal potential more negative than the dark membrane potential.

In off-centre cells the membrane potential in darkness was -46 ± 5 mV. Addition of Co^{2+} caused an average hyperpolarization of 6 mV and an average decrease in conductance of 1·5 nS. These results suggest that in darkness off-centre cells receive a tonic excitatory input which increases conductance and has a reversal potential more positive than the dark membrane potential.

4. In on-centre cells light causes a sustained depolarization. This response involves an increase in a tonic excitatory input which increases conductance and has a reversal potential more positive than the dark membrane potential.

5. In off-centre cells, light causes a sustained hyperpolarization. This response involves an increase in a sustained inhibitory input which increases conductance and has a reversal potential more negative than the dark membrane potential.

6. The depolarizing *off*-response of off-centre cells is associated with an increase in an excitatory input which increases conductance and has a reversal potential more positive than the dark membrane potential. This response may be due to a temporary increase in the excitatory input which is tonically active in darkness or may reflect an additional excitatory input.

7. It is suggested that in both on- and off-centre ganglion cells the balance of sustained excitatory and inhibitory synaptic inputs determines the resting potential

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in darkness. Centre illumination alters the balance of these inputs, by increasing one and decreasing the other, to produce the characteristic sustained light responses.

8. The possible presynaptic sources of the sustained excitatory and inhibitory inputs are discussed.

INTRODUCTION

The synaptic basis of sustained on-centre and off-centre ganglion cell responses has been the subject of a number of recent studies (Miller & Dacheux, 1976*a*, *b*; Naka, 1976, 1977; Baylor & Fettiplace, 1977; Dacheux, Frumkes, & Miller, 1979; Wunk & Werblin, 1979). These investigations have led to the widely held belief that the sustained responses of ganglion cells are due entirely to modulation of excitatory synaptic input from bipolar cells. Specifically, it is thought that on-centre ganglion cells are driven by depolarizing (on) bipolar cells via an excitatory synapse which is silent in darkness and active during illumination of the receptive field centre. Off-centre ganglion cells, on the other hand, are believed to be driven by hyperpolarizing (off) bipolar cells via an excitatory synapse whose activity is greatest in darkness and reduced during centre illumination.

Both types of ganglion cells also receive a transient inhibitory input which is only active for a short time following a change in illumination and which is thought to come from transient amacrine cells (Wunk & Werblin, 1979). Thus, except for the transient input, on-centre and off-centre ganglion cells are commonly thought of as simple followers of the two respective types of bipolar cells.

In the present paper we show that in addition to the inputs described above, both on-centre and off-centre ganglion cells in the mudpuppy retina receive sustained inhibitory synaptic input. The balance of these sustained excitatory and inhibitory inputs determines the resting membrane potential in darkness, and alterations in this balance produce the characteristic sustained responses to illumination. A preliminary account of some of the results has been given (Belgum, Dvorak & McReynolds, 1981).

METHODS

Preparation

Intracellular recordings were made from single neurones in the eyecup of the mudpuppy, *Necturus maculosus*. The dissection was performed under normal laboratory illumination. After decapitating the animal, one eye was removed and its anterior portion dissected away with fine scissors. The lens was carefully lifted out and most of the vitreous humour drawn off with filter paper. The eyecup was placed in a depression in the floor of a narrow channel in a plexiglass block and secured around its perimeter with a plastic cover slip. A continuous stream of Ringer solution flowed over the preparation at a rate of 0·5–1·0 ml/min. The composition of the Ringer was (mM): NaCl, 111; KCl, 3·0; $CaCl_2$, 1·8; glucose, 11; HEPES buffer, 5·0; adjusted to pH 7·8. A valve between the solution reservoirs and the preparation allowed changes from normal Ringer to another solution of Ringer containing 4 mM-Co^{2+} (see below) without alteration of the flow rate or fluid level in the recording chamber. The delay time for the perfusing fluid to travel from the valve to the preparation was 15–20 s, and exchange of fluid at the surface of the retina was 90 % complete within 30 s. Bathing solutions were saturated with 100 % oxygen. Experiments were performed at room temperature (20 °C). The preparation was left in darkness for 5–10 min before each experiment.

Recording

Micropipettes were made with a Livingston-type puller and filled with 4 M-potassium acetate; electrode resistance was 500–800 MΩ measured in the bathing solution. The reference electrode was

a chlorided silver wire connected to the bath by means of a Ringer–agar bridge. The recording electrode was lowered into the retina in small steps by a hydraulic microdrive. Ganglion cells were encountered within 2–10 μm after first contact was made with the retinal surface.

A high input impedance, negative capacitance preamplifier (Colburn & Schwartz, 1972) was used to record membrane potential and to inject constant currents through the recording electrode. The amplifier also contained an active bridge circuit for balancing out the voltage drop across the electrode during current injection.

Current–voltage (I–V) relations of individual ganglion cells were measured by applying steps of constant current and recording the resulting displacements of the membrane potential. In most cases, current was applied for a period of 10 s, during which time a light stimulus was presented after the membrane potential had reached a steady level. Ganglion cells in this retina have high input resistances in darkness (150–450 MΩ), which allowed I–V relations to be measured with currents of less than $\pm$0·1 nA. For the electrodes used in this study, voltage was proportional to current over the range of about $\pm$0·05 nA; non-linear properties were corrected for by measuring I–V relations of each electrode before and after each recording. In some cells, repetitive hyperpolarizing constant current pulses of short duration (100–200 ms) were applied to show the time course of conductance changes during different phases of a single response (e.g. Fig. 6).

Light stimulation

The stimulus was white light from a 45 W tungsten quartz iodine lamp operated at 6·0 V. The light passed through an electronically operated shutter, a series of calibrated neutral density filters, and a field stop which could be adjusted to give a spot of the desired size, which was projected onto the retina. A micrometer adjustment of the field stop position in two dimensions allowed exact positioning of the spot on the retina.

Calibration was accomplished in two stages. First, using a calibrated PIN diode, it was determined that the stimulator delivered $5{\cdot}15 \times 10^{13}$ quanta $\cdot$ cm^{-2} $\cdot$ s^{-1} in the plane of the retina when a 575 nm interference filter and ultraviolet blocking filter were in the light beam. 575 nm is the λ_{max} of mudpuppy cones (Liebman, 1972). Next, recordings were obtained from mudpuppy cones, which were identified by their light response and spectral sensitivity (Norman & Werblin, 1974) as well as their insensitivity to Co^{2+} (Dacheux & Miller, 1976). With the above filters in place, stimulus intensity was adjusted with neutral density filters to obtain a half-saturating response to a 70 μm diameter spot. It was consistently found that if the 575 nm filter was removed, the white light had to be attenuated by an additional 1·8 log units to produce the same response to the test flash. It follows that for mudpuppy cones the unattenuated white light was equivalent to a 575 nm light stimulus of $3{\cdot}25 \times 10^{15}$ quanta $\cdot$ cm^{-2} $\cdot$ s^{-1}.

Stimulus intensities are expressed in log units of attenuation relative to this value. Unless otherwise indicated, stimuli of constant intensity and duration were presented at 20 s intervals throughout the experiment to maintain a relatively constant state of adaptation. The spot was centred in the cell's receptive field by positioning it so as to elicit responses of maximum amplitude and minimum latency. Spot diameters ranged from 70 to 250 μm; the size of the receptive field centre of mudpuppy on-centre and off-centre ganglion cells is 500–750 μm (Karwoski & Burkhardt, 1976; Tuttle, 1977).

Identification of cell types

On-centre and off-centre ganglion cells were identified by their characteristic responses to illumination (Kuffler, 1953; Werblin & Dowling, 1969; Karwoski & Burkhardt, 1976; Tuttle, 1977). In a few cases identification was verified by injection of the dye Lucifer yellow or by antidromic stimulation of the optic nerve. Dye-injected cell bodies were in the ganglion cell layer and had axons which could be traced for 100–200 μm.

RESULTS

We shall show that both on-centre and off-centre ganglion cells receive sustained excitatory and inhibitory synaptic inputs and that these inputs determine the membrane potential in darkness and during maintained illumination. Synaptic inputs

were studied by measuring *I–V* relations under three conditions: in darkness, during illumination of the receptive field centre, and in the absence of chemically mediated synaptic input.

On-centre cells

The results described below are based on recordings from seventy on-centre cells. The average membrane potential in darkness was -56 ± 6 mV (mean $\pm$ S.D.).

Characteristic responses. The response of an on-centre cell to illumination of its receptive field centre is a maintained depolarization, which may give rise to a maintained discharge of action potentials. The depolarization and resulting action

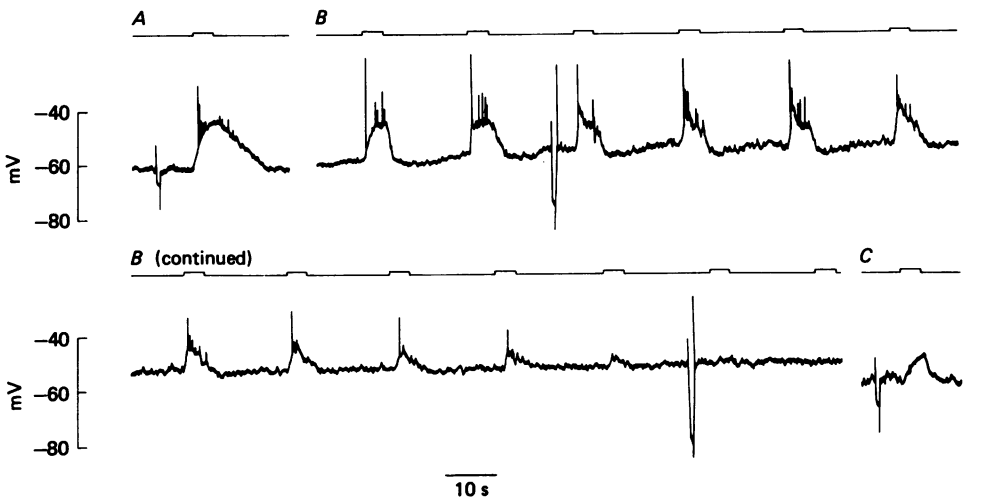

Fig. 1. Effect of Co^{2+} on an on-centre ganglion cell. Identical light stimuli (200 μm diameter spot, intensity $-4{\cdot}8$) were given every 20 s throughout the experiment. Horizontal line above responses indicates time of each light stimulus. Membrane resistance was measured by injection of a pulse of constant current ($-0{\cdot}04$ nA) at various times during the experiment. The resulting voltage displacements are proportional to input resistance at that time. *A*, control response; *B*, continuous recording of sequential responses, beginning 3 min after switching to solution containing 4 mM-Co^{2+}; *B* (continued), continuation of *B*; *C*, partial recovery 13 min after removal of Co^{2+}. In this and all subsequent Figures, responses were photographed from penwriter records and spikes are unretouched. The rise time of the penwriter (Brush 2200) was 4 ms, which typically attenuated spike amplitude by 30%.

potential discharge were graded with light intensity. At higher light intensities the action potentials decreased in amplitude and often dropped out (see Figs. 1 and 3.) This was probably due to spike inactivation since it also occurred when the cells were depolarized with current. Similar decreases in action potential firing at high light levels are seen in extracellular recordings from mudpuppy ganglion cells (Karwoski & Burkhardt, 1976), indicating that this behaviour is not due to injury of cells by electrode penetration.

Synaptic inputs in darkness. Before examining the effects of light, it was useful to understand the dark synaptic inputs. These were studied by comparing the membrane potential and conductance in the dark before and after the addition of 4 mM-cobalt

chloride to the bathing medium. Cobalt is a competitive inhibitor of Ca^{2+} movement through voltage-dependent calcium channels (Hagiwara & Takahashi, 1967). A concentration of 4 mM-Co^{2+} was used to ensure that most of the calcium influx into presynaptic terminals was blocked, which reduces transmitter release to a minimum (Weakly, 1973). As will be shown below, the effects of such treatment were readily reversed suggesting that Co^{2+} is removed from its blocking site and that it probably does not accumulate intracellularly in presynaptic terminals or in post-synaptic cells. It is possible that cobalt blocks any steady-state calcium current into ganglion cells and, because of this, might block any steady-state calcium-activated potassium current, but there is no evidence in these or any other cells that such currents contribute to the resting potential. In studies of other neurones in the vertebrate retina the effects of Co^{2+} have been interpreted as due to the blocking of chemical transmission (Cervetto & Piccolino, 1974; Kaneko & Shimazaki, 1975; Dacheux & Miller, 1976; Marshall & Werblin, 1978; Wu & Dowling, 1978).

In the on-centre cell shown in Fig. 1, the addition of Co^{2+} caused depolarization of the membrane potential, a large increase in input resistance, and disappearance of the light response. The increase in noise appears to be due to a voltage-dependent property of the membrane since it disappeared when membrane potential was hyperpolarized by the extrinsic current pulses used to measure input resistance. *I–V* relations were determined by passing steps of constant current across the membrane and measuring the resulting potential change 1 s after the onset of the current pulse, at which time the potential had reached a steady value. Fig. 2 shows *I–V* relations for this cell made in darkness before application of Co^{2+}, and in Co^{2+} after all light-evoked responses were blocked and membrane potential and resistance had reached new steady levels. In this cell, when membrane potential was depolarized by more than 25 mV there was a large increase in slope conductance. This behaviour was typical although the potential level at which it occurred varied in different cells. Comparisons of the *I–V* relations were always made at less positive membrane potentials. In this cell, the reciprocal of the slope of the line drawn through the data points measured in darkness (without Co^{2+}) gives a resting conductance of 2·9 nS (i.e. an input resistance of 340 MΩ). In Co^{2+} the cell depolarized by 14 mV and input conductance decreased to 1·4 nS. Data from thirteen on-centre cells are summarized in Table 1: addition of 4 mM-Co^{2+} caused a mean depolarization of 10 mV and a mean conductance decrease of 1·9 nS. These results indicate that in darkness on-centre cells receive tonic synaptic input, the net effect of which is to increase conductance and hyperpolarize the membrane potential. These experiments do not rule out the possibility that more than one kind of synaptic input is active in darkness. However, even if that were the case, the dominant input must be one which has these properties.

Synaptic basis of the light response. Fig. 3*A* shows the light response of the on-centre cell described above at different levels of membrane potential, produced by the application of steady polarizing current. The response increased in amplitude when the cell was hyperpolarized and decreased when it was depolarized. Current–voltage relations during the depolarizing light response were measured after the response had reached a plateau level; this procedure ensured that contamination from the transient i.p.s.p. at the onset of light (see Wunk & Werblin, 1979) was minimal. These measurements are shown in Fig. 3*B*, together with the *I–V* relations measured in

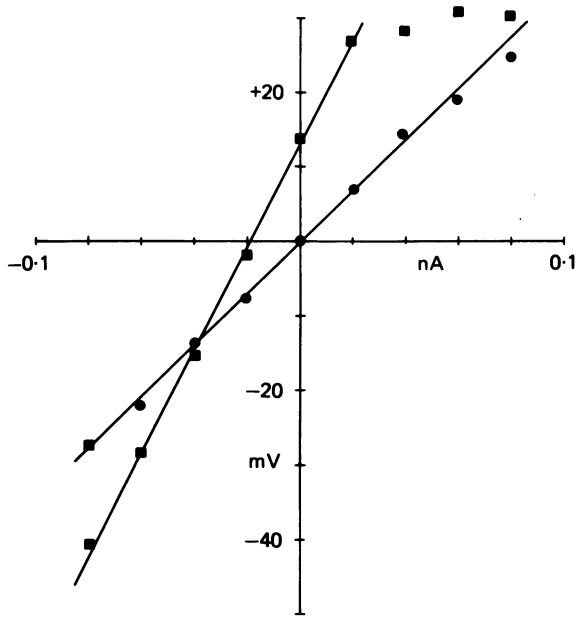

Fig. 2. Effect of Co^{2+} on current–voltage relations of an on-centre ganglion cell. This is the same cell shown in Fig. 1. Measurements made in darkness (●) and after synaptic transmission had been blocked with 4 mM-Co^{2+} (■). Membrane potential is plotted relative to resting potential in darkness, which was −62 mV. The cell was lost 13 min after removal of Co^{2+}, at which time membrane potential had recovered to −58 mV and conductance had increased to 2·3 nS. This is cell M in Table 1.

TABLE 1. Effect of Co^{2+} on membrane conductance and membrane potential of on-centre ganglion cells

Cell	R (MΩ)	G (nS)	G_{Co} (nS)	$G-G_{Co}$ (nS)	V (mV)	V_{Co} (mV)	$V-V_{Co}$ (mV)
A	240	4·2	1·9	−2·3	−59	−39	+20
B	350	2·9	1·3	−1·6	*	*	+8
C	150	6·7	3·3	−3·4	−62	−62	0
D	320	3·1	1·8	−1·3	−51	−41	+10
E	240	4·2	2·4	−1·8	−68	−48	+20
F	150	6·7	2·2	−4·5	−50	−45	+5
G	180	5·6	2·5	−3·1	−47	−43	+4
H	220	4·5	2·9	−1·6	−55	−43	+4
I	250	4·0	3·3	−0·7	*	*	+20
J	400	2·5	2·0	−0·5	−48	−48	0
K	275	3·6	2·3	−1·3	−61	−53	+8
L	300	3·3	2·3	−1·0	−69	−61	+8
M	340	2·9	1·4	−1·5	−62	−48	+14
	263±78	4·2±1·4	2·3±0·6	1·9±1·2	57±8	48±8	10±7

Abbreviations as follows: R, input resistance in darkness; G, membrane conductance ($= 1/R$) in darkness; G_{Co}, membrane conductance in presence of 4 mM-Co^{2+}; V, resting membrane potential in darkness; V_{Co}, membrane potential in presence of Co^{2+}; * indicates cells in which it was not possible to accurately determine absolute membrane potential in darkness. Bottom row indicates mean ± S.D.

darkness and in Co^{2+} (from Fig. 2). Light caused a depolarization of 23 mV and an increase in slope conductance from the dark value of 2·9 nS to 5·0 nS. Again, there was a marked increase in slope conductance when membrane potential was depolarized.

It was argued in the preceding section that membrane potential in darkness was hyperpolarized by a synaptic input which increased conductance. Comparison of the *I–V* relations measured in light and in Co^{2+} shows that in light the cell was

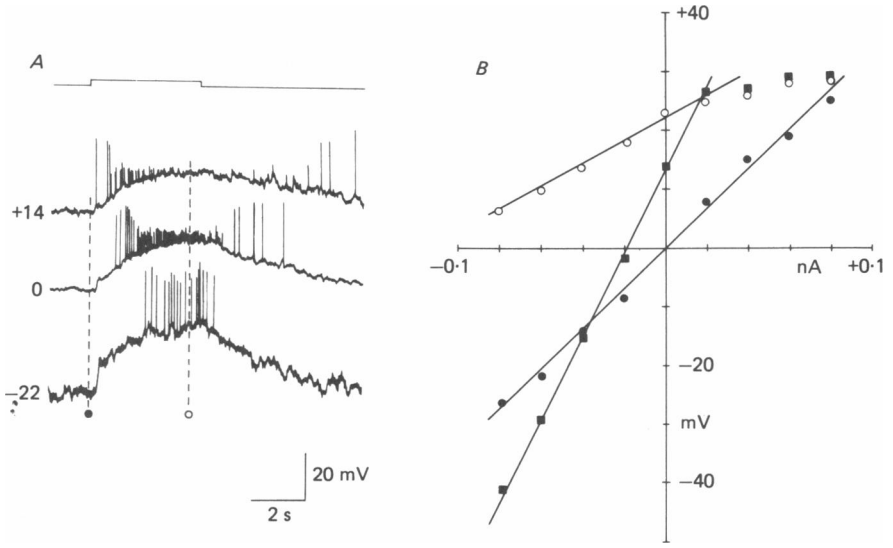

Fig. 3. Current–voltage relations of an on-centre cell in which light caused a net increase in conductance. *A*, responses to identical light stimuli (200 μm diameter spot, intensity −4·8) at three different levels of membrane potential, indicated at left of each trace. Onset of polarizing current step was 2 s before beginning of records. Dashed lines indicate times at which *I–V* measurements were made in darkness and during the sustained light response. In this and all subsequent Figures, when there were irregular fluctuations in membrane potential in darkness the potential was averaged over the 1 s period preceding the time indicated by the vertical line. *B*, *I–V* relations for this cell measured at the times indicated in *A* in darkness (●), during the sustained light response (○), and when synaptic inputs were blocked with 4 mM-Co^{2+} (■). For this and subsequent *I–V* relations, straight lines are drawn through the linear portions of the *I–V* relations. Resting potential was −62 mV. This is cell M in Table 1.

depolarized by a synaptic input which also increased conductance. Therefore, the light response represents a change from inhibition to excitation. Since the conductance in light is greater than in darkness, it follows that light caused an increase in excitation. However, the effect of light on the inhibitory input cannot be determined from these data. Furthermore, since the excitatory input may be active at reduced levels in darkness it is not possible to determine the absolute changes in either input, or their reversal potentials, from the *I–V* relations. Thus, these results indicate the *net* effects of synaptic input in darkness and in light. The results described next, however, suggest that the light response involves both an increase in excitation and a decrease in inhibition.

The results shown in Fig. 4 are from a cell in which the conductance during the

light response was the same as in darkness. The amplitude of the sustained light response did not change significantly when the cell was depolarized or hyperpolarized by extrinsic current, and the *I–V* relations measured in darkness and in light had the same slope, which corresponds to a conductance of 3·3 nS. In the presence of Co^{2+} the cell depolarized to a potential midway between that in darkness and that in light,

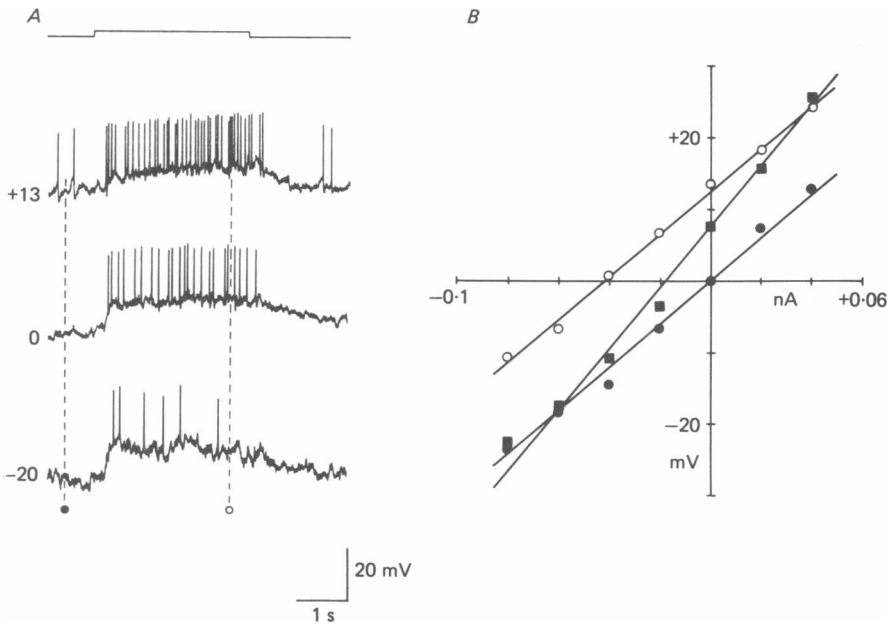

Fig. 4. Current–voltage relations of an on-centre ganglion cell in which light caused no net change in conductance. *A*, responses to identical light stimuli (200 μm diameter spot, intensity −4·8) at three different levels of membrane potential. Details as in Fig. 3. *B*, *I–V* relations measured at the times indicated in *A* in darkness (●), in light (○), and in the presence of Co^{2+} (■). Resting potential was −69 mV. This is cell L in Table 1.

and conductance decreased to a value of 2·3 nS. Comparison of the dark and Co^{2+} *I–V* relations shows that there was a net inhibitory input in darkness which hyperpolarized the cell and increased conductance. In light there was a net excitatory input which depolarized the cell and also increased conductance relative to the value in Co^{2+}. These results imply that light caused a simultaneous decrease in inhibition and an increase in excitation; in this cell the separate conductance changes due to the two inputs were of equal magnitude, so that the light-evoked depolarization was associated with no net change in conductance.

Fig. 5 shows results from an on-centre cell in which the depolarizing light response was associated with a net *decrease* in conductance relative to the value in darkness. In this cell, the amplitude of the depolarizing light response decreased when the cell was hyperpolarized and increased when it was depolarized. Light caused a depolarization of 12 mV which was associated with a conductance decrease of 1·0 nS relative to the value in darkness. However, comparison of the dark and light *I–V*

relations with those obtained in Co^{2+} reveals that in darkness there was net inhibition and in light there was net excitation, both of which were associated with increases in conductance. In this cell, light decreased the inhibitory input more than it increased the excitatory input, so that the resulting depolarization was associated with a net decrease in conductance.

Of the on-centre cells studied, 80 % (fifty-six of seventy) showed a net conductance increase during the sustained light response, while the remaining 20 % (fourteen of seventy) showed either no change or a net decrease in conductance. For a given cell,

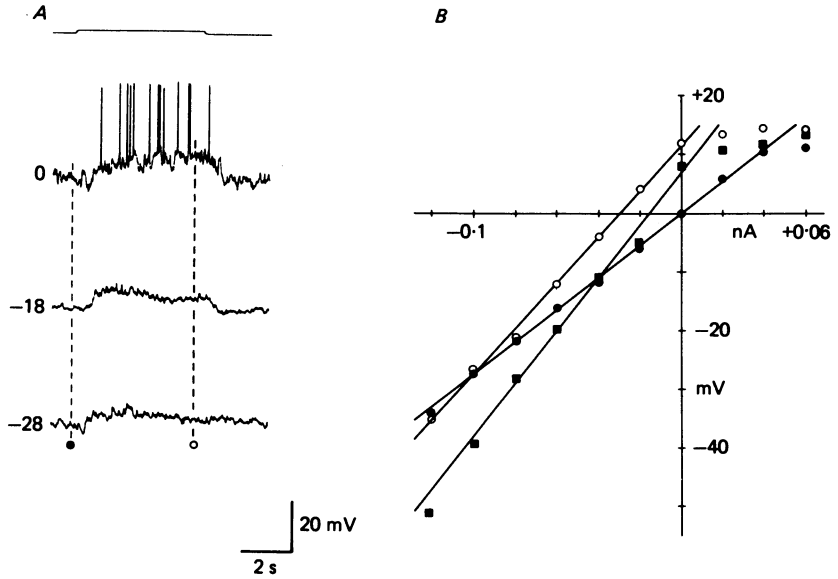

Fig. 5. Current–voltage relations of an on-centre ganglion cell in which light caused a net decrease in conductance. *A*, responses to identical light stimuli (250 μm diameter spot, intensity −3·6) at three different levels of membrane potential. Details as in Fig. 3. *B*, *I–V* relations measured at the times indicated in *A* in darkness (●), in light (○) and in the presence of Co^{2+} (■). Resting potential was −61 mV. This is cell K in Table 1.

the type of conductance change (i.e. increase, decrease, or no change) was the same for all light intensities and response amplitudes. In spite of the different types of conductance changes observed in going from darkness to steady illumination, the responses of all on-centre cells can be explained by a common mechanism. Comparison of the dark and light *I–V* relations with those measured in the absence of synaptic input, rather than only with each other, indicates that these cells receive a net inhibition in darkness and a net excitation in light. Both the excitatory and inhibitory inputs act via conductance-increase mechanisms. During illumination the excitatory input is increased and the inhibitory input may be decreased, but the relative amounts by which the two inputs change is variable, so that the resulting depolarization may be accompanied by either a net increase, a net decrease, or no change in conductance.

Off-centre cells

Recordings were made from fifty-six off-centre cells. The average membrane potential in darkness was -46 ± 5 mV (mean $\pm$ S.D.).

Characteristic responses. Off-centre ganglion cells responded to centre illumination with a sustained hyperpolarization which was maintained for the duration of the light stimulus (Fig. 6*A*). At stimulus onset the hyperpolarization was rapid and could

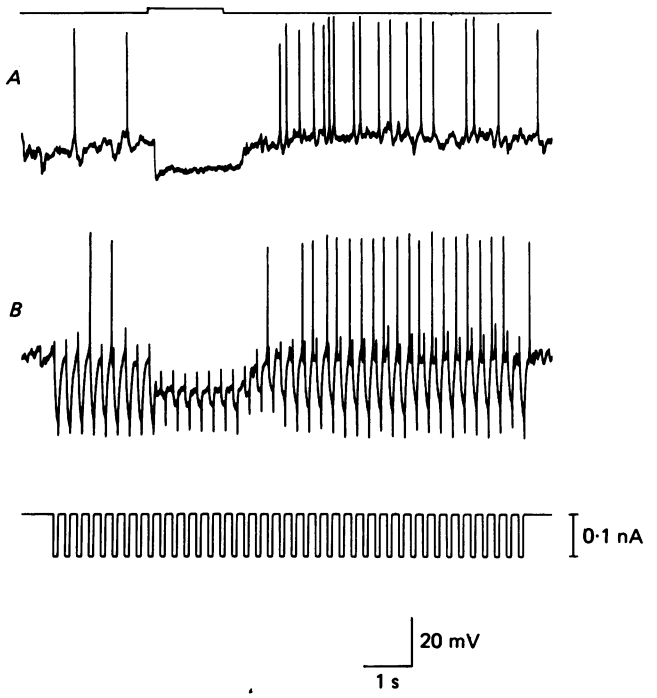

Fig. 6. Time course of light-evoked conductance changes in an off-centre ganglion cell. *A*, response to a 70 μm diameter spot, intensity $-2{\cdot}4$. *B*, response to identical light stimulus as above with superimposed $-0{\cdot}1$ nA constant current pulses. Current intensity shown in lower trace. Voltage displacement caused by each current pulse is proportional to input resistance of cell at that time. Large, brief transients at onset and termination of each current pulse are capacitative artifacts. Resting potential was -56 mV. This is cell F in Table 2.

transiently exceed the sustained level. This is due in part to a separate, transient inhibitory input (see Wunk & Werblin, 1979) which will be discussed in detail in a subsequent paper (J. H. Belgum, D. R. Dvorak & J. S. McReynolds, in preparation). During the maintained hyperpolarization the membrane noise was of much lower amplitude than in the dark. At the termination of the light stimulus the cells usually depolarized to a level more positive than the membrane potential in the dark, which triggered a burst of action potentials. The duration of this *off*-response was typically 3–10 s but could be as long as 30 s.

Light-induced changes in conductance. Fig. 6*B* shows the changes in conductance associated with the centre response of an off-centre cell, measured by passing brief

hyperpolarizing constant current pulses across the cell membrane. The amplitude of the resulting voltage displacements are proportional to the cell's input resistance. Both the sustained light-evoked hyperpolarization and the depolarizing *off*-response were associated with increases in conductance relative to the dark level, which indicates that these two components of the response cannot be due to modulation of a single synaptic input. The even larger conductance increase seen shortly after the

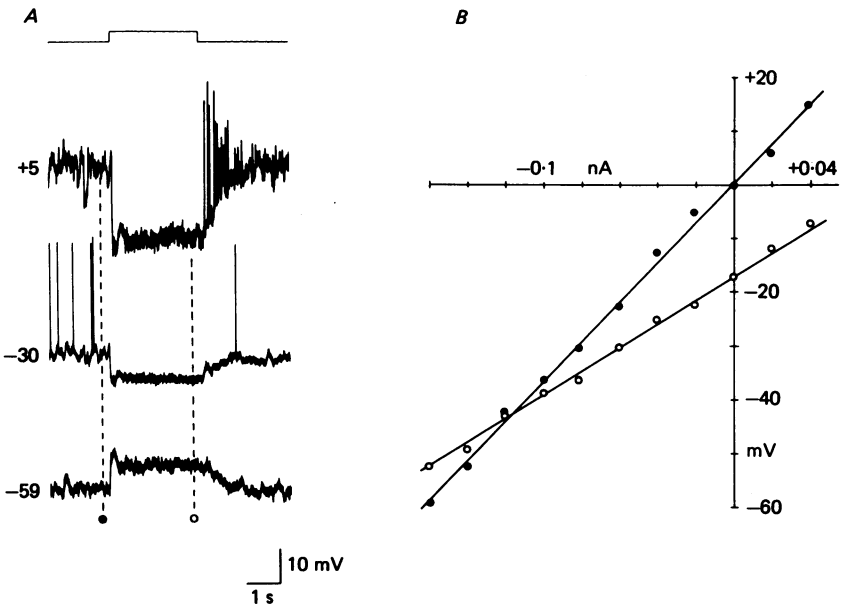

Fig. 7. Current–voltage properties of an off-centre ganglion cell. *A*, responses to identical light stimuli (250 μm diameter spot, intensity −3·6) at three different levels of membrane potential. Details as in Fig. 3. *B*, *I–V* relations measured at the times indicated in *A* in darkness (●) and during illumination (○). Resting potential was −40 mV.

onset of the light stimulus is due to the transient inhibitory input mentioned earlier. The sustained hyperpolarization and conductance increase were maintained with light stimuli of up to 45 s (the longest stimulus duration in which conductance was measured). In contrast to the variability of the conductance changes associated with sustained responses of on-centre cells, the light-evoked hyperpolarization of off-centre cells was *always* accompanied by a significant increase in conductance relative to the dark level. Fig. 7*A* shows the centre response of an off-centre cell at different levels of membrane potential. The light-evoked sustained hyperpolarization became larger when the cell was depolarized with extrinsic current, and it was clearly reversed in polarity when the cell was sufficiently hyperpolarized. The *I–V* relations for this cell (Fig. 7*B*) show that the conductance in darkness was 2·8 nS; light caused a maintained hyperpolarization of 18 mV and increased conductance to 4·5 nS.

Results from another off-centre cell which had a more prominent *off*-depolarization are shown in Fig. 8. In this cell a large voltage-dependent conductance increase was present with depolarization of more than 10 mV relative to the dark potential; similar

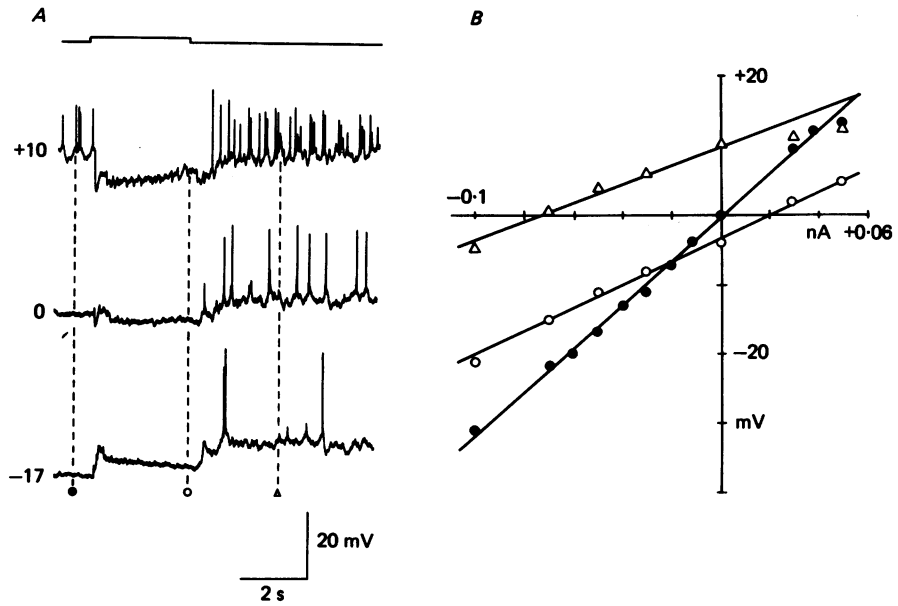

Fig. 8. Current–voltage properties of an off-centre ganglion cell. *A*, responses to identical light stimuli (200 μm diameter spot, intensity −4·8) at three different levels of membrane potential. Details as in Fig. 3. *B*, *I–V* relations measured at the times indicated in *A* in darkness (●), in light (○), and during the *off*-depolarization (△). Resting potential was −50 mV.

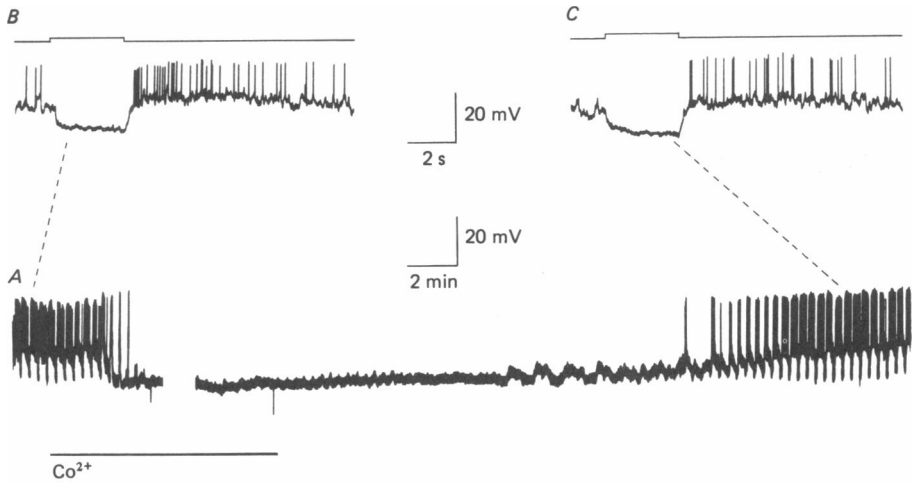

Fig. 9. Effect of Co^{2+} on an off-centre ganglion cell. *A*, recording at slow chart speed showing time course of Co^{2+} effect and recovery. Identical light stimuli (200 μm diameter spot, intensity −3·6) were given every 20 s throughout the experiment. During the time indicated by the horizontal line the Ringer solution contained 4 mM-Co^{2+}. The break in the record represents a 60 s period during which the *I–V* relation shown in Fig. 10 was measured. *B* and *C*, responses made at a faster chart speed before and after Co^{2+}. Duration of light stimulus indicated above responses. Resting potential was −39 mV. This is cell K in Table 2.

rectification was typical of most off-centre cells. The linear portions of the *I–V* relations show that the light-evoked hyperpolarization was associated with a 3·2 nS increase in conductance relative to the value in darkness. The *I–V* relation measured during the *off*–depolarization shows that this part of the response was also associated with a conductance increase relative to darkness, suggesting that it results from an increase in excitatory synaptic input. Since this response occurred even when the preceding light response was reversed in polarity (Fig. 8*A*, bottom trace) it cannot be accounted for by voltage-dependent membrane properties. Furthermore, such responses did not occur following the termination of hyperpolarizing current pulses.

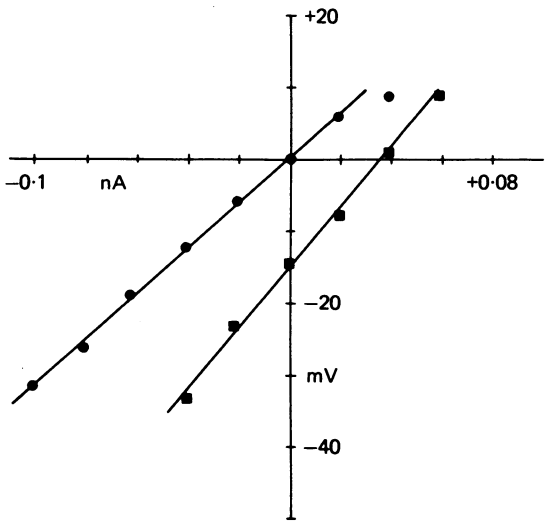

Fig. 10. Effect of Co^{2+} on the *I–V* relations of an off-centre ganglion cell. Data are from the cell shown in Fig. 9. The *I–V* relation in darkness (●) was measured just before the beginning of record *A* in Fig. 9, and the *I–V* relation in Co^{2+} (■) was measured during the tie indicated by the break in that record. Resting potential was −39 mV.

TABLE 2. Effect of Co^{2+} on membrane conductance and membrane potential of off-centre ganglion cells

Cell	R (MΩ)	G (nS)	G_{Co} (nS)	$G-G_{Co}$ (nS)	V (mV)	V_{Co} (mV)	$V-V_{Co}$ (mV)
A	300	3·3	2·0	−1·3	−45	−45	0
B	160	6·3	2·1	−4·2	−48	−52	−4
C	200	5·0	4·2	−0·8	*	*	−5
D	130	7·7	5·6	−2·1	−41	−47	−6
E	300	3·3	2·8	−0·5	−45	−53	−8
F	250	4·0	2·5	−1·5	−56	−56	0
G	250	4·0	2·0	−2·0	−42	−51	−9
H	150	6·7	5·0	−1·7	−45	−55	−10
I	250	4·0	2·9	−1·1	−54	−47	−3
J	150	6·7	4·2	−2·5	−43	−46	−3
K	310	3·2	2·4	−0·8	−39	−54	−15
	222±67	4·9±1·6	3·2±1·3	−1·7±1·0	46±5	52±4	−6±5

Abbreviations as in Table 1. Bottom row indicates mean ± S.D.

Synaptic inputs in darkness. The question of whether off-centre cells receive a tonic excitatory input in darkness, as postulated by previous investigators, was examined by blocking synaptic activity in darkness with cobalt. As shown in Fig. 9, in the presence of Co^{2+} the cell hyperpolarized by 15 mV and the light response disappeared. *I–V* relations for this cell measured in darkness and when transmission had been blocked by Co^{2+} are shown in Fig. 10. In darkness, the conductance was 3·2 nS, and

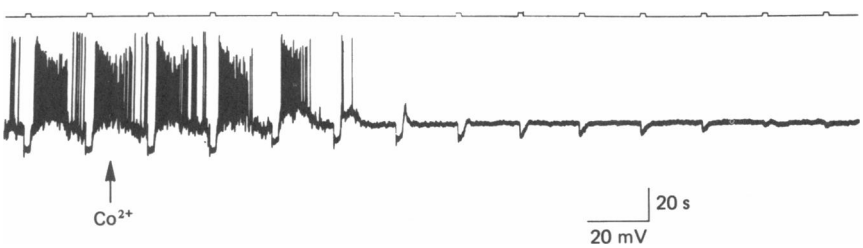

Fig. 11. Effect of Co^{2+} on an off-centre ganglion cell in which blocking synaptic input caused no change in membrane potential. Identical light stimuli (70 μm diameter spot, intensity −2·4) were given every 20 s throughout the recorded period; stimulus markers are shown in upper trace. At the time indicated by the arrow, the bathing solution was switched to one containing 4 mM-Co^{2+}. *I–V* measurements made just before and after the period shown in this recording showed that Co^{2+} caused conductance to decrease from 4·0 to 2·5 nS. Other experimental manipulations were performed while the cell was still in Co^{2+}. When Co^{2+} was later washed out there was recovery of both the light response and the resting membrane conductance. Resting potential was −56 mV. This is cell F in Table 2.

the hyperpolarization in Co^{2+} was associated with a decrease in conductance to 2·4 nS. Table 2 summarizes results from eleven off-centre cells: blocking synaptic input with Co^{2+} caused a mean hyperpolarization of 6 mV and a mean conductance decrease of 1·7 nS. These results indicate that in darkness off-centre cells receive tonic synaptic input, the net effect of which is to increase conductance and depolarize membrane potential.

In a few cells, such as the one illustrated in Fig. 11, Co^{2+} blocked the light response and increased membrane resistance without causing a change in membrane potential. Although not shown here, *I–V* measurements for this cell made before and after the light response was blocked showed that in the presence of Co^{2+} conductance was decreased by 1·7 nS. Both the light response and conductance recovered when Co^{2+} was removed. These results could be explained if it is assumed that both excitation and inhibition contribute to the membrane potential in darkness.

Fig. 12 shows the *I–V* relations for a single cell measured in darkness, during steady illumination, during the off-depolarization and in Co^{2+}. As can be seen by comparing the dark and Co^{2+} *I–V* relations, the dominant synaptic input in darkness was excitatory. Comparison of the light and Co^{2+} *I–V* relations shows that during illumination the membrane was hyperpolarized by 9 mV. Because the conductance in light was increased relative to that in darkness it is evident that light increased the inhibitory input, but its effects on the excitatory input cannot be determined from this data. The intersection of the light and dark *I–V* relations indicates the reversal potential of the hyperpolarizing *response* relative to the dark potential, and

the intersection of the light and Co^{2+} *I–V* relations indicates the reversal potential for the *total* synaptic activity present in light. Neither of these reversal potentials are necessarily that of the inhibitory mechanism itself, since it is not known how the excitatory input is affected by light. The depolarizing *off*-response has an apparent reversal potential more positive than the dark potential. Again, since more than one synaptic input may change during this response, the apparent reversal potential of this phase of the response may not be that of a single mechanism. Whether this excitatory input is distinct from that present in darkness cannot be determined from these experiments.

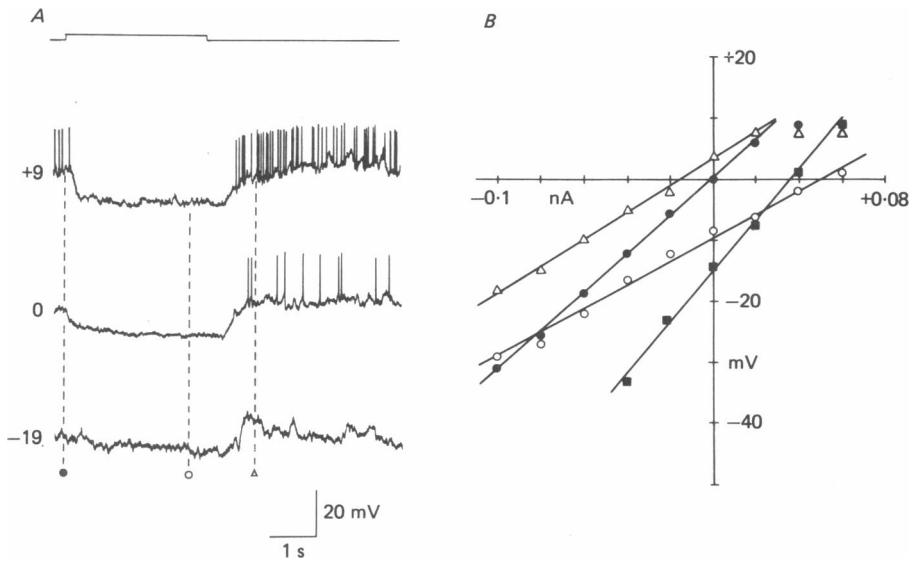

Fig. 12. *I–V* relations during different phases of the response of an off-centre ganglion cell. *A*, responses to identical light stimuli (200 μm diameter spot, intensity −3·6) at three different levels of membrane potential. Details as in Fig. 3. *B*, *I–V* relations made at the times indicated in *A* in darkness (●), in light (○), during the depolarizing off-response (△), and when synaptic input was blocked with Co^{2+} (■). Resting potential was −39 mV.

DISCUSSION

These experiments demonstrate that on- and off-centre ganglion cells of the mudpuppy retina receive sustained excitatory *and* inhibitory synaptic inputs, both of which act by increasing membrane conductance. Comparison of Tables 1 and 2 shows that in the absence of synaptic input the average membrane potential of on- and off-centre cells is about the same, but when transmission is intact the average resting potential in darkness of on-centre cells is hyperpolarized, and that of off-centre cells depolarized, relative to this value. In on-centre cells illumination increases excitatory input and in off-centre cells it increases inhibitory input. It is likely that light also decreases the input which was dominant in darkness, although this cannot be proven unequivocally with present techniques. Similarly, the input which is dominant in light may be reduced, but not silent, in darkness. In summary, membrane

potential in darkness appears to be determined by the balance of two opposing sustained inputs, and illumination of the receptive field centre seems to alter this balance to produce the characteristic sustained light response of each cell type. Our results suggest that sustained responses of on- and off-centre ganglion cells are not caused only by modulation of excitatory input from bipolar cells, as generally supposed, but are determined by the combined effects of excitation and inhibition acting in a push–pull manner. The reversal potentials for the excitatory and inhibitory inputs themselves can not be determined in these experiments because any manipulation, either by light or by cobalt, may cause changes in both inputs. Nevertheless, it can be concluded that each cell type receives two separate sustained inputs which are modulated to produce the sustained light responses.

Voltage-dependent conductance changes were observed in most cells when the membrane potential was depolarized by more than a few millivolts, and it is probable that with sufficient depolarization such behaviour may be typical of all on- and off-centre ganglion cells. Since the voltage-dependent conductance increases can be larger than that produced by synaptic action, they could lead to large errors in interpreting synaptically-produced conductance changes. For example, it has been reported that the sustained hyperpolarizing light response of off-centre ganglion cells is due to a decrease in conductance (Dacheux *et al.* 1979). However, that conclusion was based on measurements of conductance with depolarizing current pulses only, and may be complicated by voltage-dependent processes.

It is thought that the excitatory input to on- and off-centre cells derives from depolarizing and hyperpolarizing bipolar cells, respectively (Miller & Dacheux, 1976*a*, *b*; Naka, 1976, 1977; Baylor & Fettiplace, 1977; Dacheux *et al.* 1979; Wunk & Werblin, 1979). The source of the sustained inhibitory input is unknown, but if we assume that transmitter release is caused by depolarization of presynaptic terminals, predictions can be made regarding the response properties of the presynaptic cells. For on-centre cells, inhibition is dominant in darkness and reduced during centre illumination; the presynaptic cell(s) which release the inhibitory transmitter should therefore be centre-hyperpolarizing. For off-centre cells, inhibition is strongest during centre illumination and is reduced in darkness; the presynaptic cell(s) mediating this inhibition should be centre-depolarizing. In either case, the sustained inhibitory input would have to come from a cell whose response was sustained and of opposite polarity to that of the ganglion cell receiving the input.

As noted above, bipolar cells are generally regarded as providing only excitatory input to ganglion cells, although it has recently been reported that glycine, a transmitter commonly associated with inhibition, is taken up by certain types of bipolar cells in cat retina (McGuire, Stevens & Sterling, 1980). Studies of the distribution of terminals in the inner plexiform layer suggest that in some species bipolar cells of one polarity are not likely to synapse onto the opposite type of ganglion cell (Famiglietti, Kaneko & Tachibana, 1977; Nelson, Famiglietti & Kolb, 1978), although in other species this segregation of terminals may not be as strict (Davis & Naka, 1980; Famiglietti, 1981; Weiler & Marchiafava, 1981). Since such studies have not been performed in mudpuppy we cannot rule out the possibility that a class of bipolar cells could provide the sustained inhibitory input. On the other hand, there is autoradiographic and histochemical evidence from many species that the

inhibitory transmitters glycine and γ-aminobutyric acid (GABA) are present in separate classes of amacrine cells (Marshall & Voaden, 1974; Marc, Stell, Bok & Lam, 1978; Pourcho, 1980; Marc & Lam, 1981). We shall present evidence in a later paper that the sustained inhibitory input to mudpuppy ganglion cells may be mediated by GABA. Hyperpolarizing and depolarizing sustained amacrine cells have been described in teleost retina (Kaneko, 1973; Naka & Ohtsuka, 1975; Chan & Naka, 1976; Murakami & Shimoda, 1977), and we have encountered cells with similar responses in mudpuppy retina. The role of these cells is not known, but it may be that one of their functions is to provide the sustained inhibitory input.

Although the results presented in this study are the first direct demonstration of sustained inhibitory input to vertebrate retinal ganglion cells, two previous studies based on extracellular recordings have suggested that such an input should exist. Enroth-Cugell & Pinto (1972) have proposed a model which accounts for centre responses of off-centre cells and surround responses of on-centre cells in the cat retina in terms of overlapping excitatory and inhibitory processes with different time courses. Levine & Shefner (1977) studied the variability of spike discharges in goldfish retinal ganglion cells and proposed a model which accounts for ganglion cell responses in terms of independent excitatory and inhibitory processes which interact at the ganglion cell level. It remains to be seen whether sustained inhibition in retinal ganglion cells can be demonstrated in other species, and to what extent our findings represent a more general feature of retinal organization.

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