

## LIGHT-EVOKED SUSTAINED INHIBITION IN MUDPUPPY RETINAL GANGLION CELLS

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**Abstract**—Intracellular recordings were made from off-center ganglion cells in the retina of the mudpuppy, *Necturus maculosus*. Current–voltage measurements revealed that the sustained light-evoked hyperpolarization of these cells is due to a sustained inhibitory synaptic input with a reversal potential more negative than the resting potential.

### INTRODUCTION

Many investigators have concluded that the sustained responses and basic receptive field properties of off-center retinal ganglion cells are determined by modulation of an excitatory synaptic input from hyperpolarizing bipolar cells (Miller and Dacheux, 1976; Naka, 1976, 1977; Baylor and Fettiplace, 1977; Wunk and Werblin, 1979). It has been inferred that the excitatory synapse is tonically active in darkness, when the hyperpolarizing bipolar cells are depolarized, and that the activity of the synapse is reduced during center stimulation, when these bipolar cells are hyperpolarized. Off-center ganglion cells also receive an inhibitory input (presumably from transient amacrine cells) which is active for a brief period following a change in illumination (Wunk and Werblin, 1979).

In the present paper we report that off-center ganglion cells in the mudpuppy retina receive a third type of synaptic input which is inhibitory and whose activity is sustained during prolonged center stimulation. Our study indicates that the sustained responses of these ganglion cells are due to changes in sustained excitatory and inhibitory synaptic inputs.

### METHODS

Intracellular responses were recorded from off-center ganglion cells in superfused mudpuppy eyecups using micropipettes filled with 4 M potassium acetate. Electrode resistance, measured in the bathing solution, ranged from 400–800 M $\Omega$ . A high input impedance preamplifier with capacity compensation (Colburn and Schwartz, 1972) was used to record membrane potential and to inject constant current through the recording electrode. An active bridge circuit was used to balance out the voltage drop across the electrode resistance during current injection. For the electrodes used in this study, voltage was proportional to current over the range of about  $\pm 0.05$  nA; nonlinear properties were evident with greater currents and were corrected for by measuring the current–voltage

properties of the electrode before and after each recording.

The stimulus was a spot of white light (70–200  $\mu$ m dia) centered in the cell's receptive field. The size of the stimulus was small relative to the size of the receptive field center, which is 500–750  $\mu$ m in diameter (Karwoski and Burkhardt, 1976). Stimuli were typically 2–5 sec duration and were presented every 20 sec. The unattenuated light stimulus at the retina was the photopic equivalent of  $3.25 \times 10^{15}$  photons  $\cdot$  cm $^{-2}$   $\cdot$  sec $^{-1}$  at 575 nm, the  $\lambda_{\max}$  of mudpuppy cones (Liebman, 1972). Surround antagonism could be evoked with appropriate stimuli but will not be discussed in this report.

### RESULTS

The response of an off-center ganglion cell to illumination of its receptive field center is a sustained hyperpolarization which persists for the duration of the light stimulus (Fig. 1a). At light on, the hyperpolarization is rapid and transiently exceeds the maintained level. At light off, the cell depolarizes beyond the previous dark level and generates a burst of action potentials. The changes in resistance which occur during the light response are illustrated in Fig. 1b. Resistance was measured by injecting hyperpolarizing constant current pulses (–0.1 nA) through the recording electrode. The amplitude of the resulting voltage displacement is proportional to the input resistance of the cell at that time. In darkness the input resistance of this cell was 280 M $\Omega$ . Throughout the entire hyperpolarizing light response the resistance was only 100 M $\Omega$ , which corresponds to a conductance increase of 6.4 nS. Note that conductance was also elevated, relative to darkness, during the off-depolarization. Similar measurements were made in 31 cells and in every case the sustained hyperpolarization was associated with a maintained increase in conductance, even with light stimuli as long as 30 sec in duration.

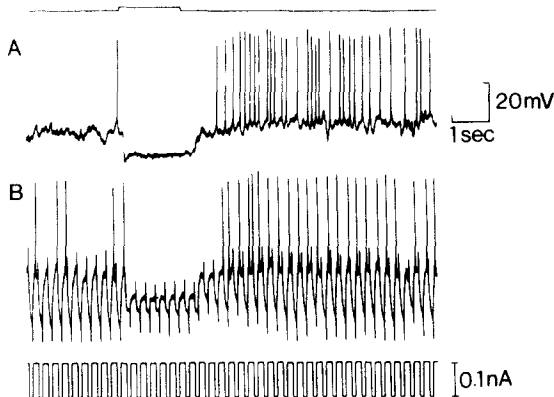
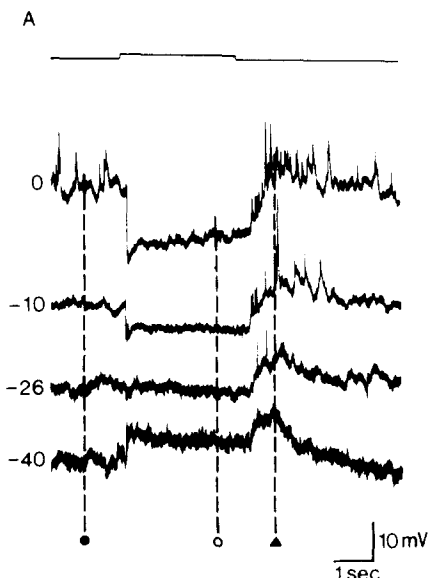


Fig. 1. Time-course of light-evoked resistance changes in an off-center ganglion cell. (A) Response to a  $70\ \mu\text{m}$  dia spot centered in the receptive field, intensity  $-2.4$  log units. (B) Response to identical light stimulus as above with superimposed  $-0.1$  nA constant current pulses. Current intensity shown in the lower trace. Voltage displacement caused by each pulse is proportional to the input resistance of the cell at that time.

The effect of steady hyperpolarizing current on the response of another off-center ganglion cell is shown in Fig. 2a. Constant current steps, 10 sec in duration, were applied through the recording electrode, and the light stimulus was presented 2 sec after the onset of each step. The normal response (zero current) is shown in the upper trace: membrane potential in darkness was  $-54$  mV, and all potentials are indicated relative to this value. Notice that as the cell is polarized to more negative potentials, the sustained hyperpolarization decreases in amplitude and eventually reverses polarity, as is clearly seen in the



bottom trace. The transient phase of the hyperpolarizing response behaves similarly, while the off-depolarization steadily increases in amplitude. The current-voltage ( $I$ - $V$ ) relations of this cell are shown in Fig. 2b. In this example, depolarization by more than 15 mV relative to the dark potential causes a large decrease in resistance. At less positive potentials, where this voltage-dependent process is inactive, the data points fall on straight lines. The  $I$ - $V$  relation measured in steady darkness (filled circles) has a slope resistance of  $250\ \text{M}\Omega$ . Center stimulation causes a maintained hyperpolarization of 13 mV, and the  $I$ - $V$  relation for this part of the light response (open circles) has a slope resistance of  $130\ \text{M}\Omega$ ; thus light caused a conductance increase of  $3.7\ \text{nS}$  relative to steady darkness. The reversal potential for the sustained hyperpolarizing response is 28 mV more negative than the normal dark potential. The  $I$ - $V$  relation for the off-depolarization (triangles) has a slope resistance of  $200\ \text{M}\Omega$ . The extrapolated potential at which the depolarizing off-response reverses polarity is more positive than the dark potential, but actual reversal cannot be demonstrated because of the voltage-dependent conductance increase.

#### DISCUSSION

These results show that the light-evoked sustained hyperpolarization of mudpuppy off-center ganglion cells is due to the action of an inhibitory synaptic input which increases conductance and which has a reversal potential more negative than the membrane potential in darkness. Further, the study provides evidence that the excitatory synaptic input to these cells

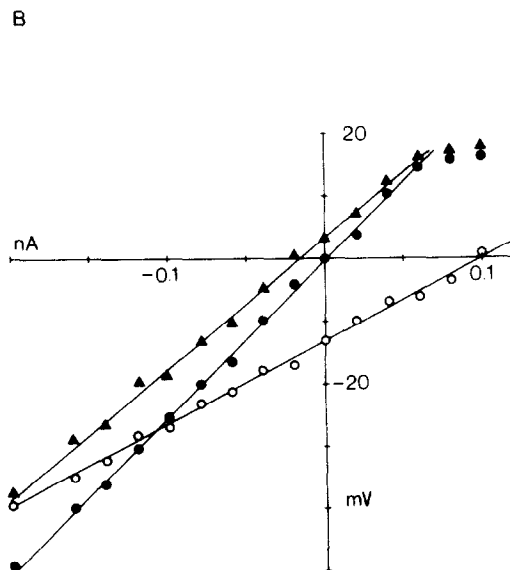


Fig. 2. Current-voltage relations of an off-center ganglion cell. (A) Responses to identical light stimuli ( $200\ \mu\text{m}$  dia spot, intensity  $-7.2$  log units) at four different membrane potentials. Numbers at left of each trace indicate membrane potential relative to resting dark potential. Onset of each polarizing current step was 1.5 sec before beginning of records. (B)  $I$ - $V$  relations for this cell measured at times indicated in (A) in darkness (filled circles), during the light-evoked sustained hyperpolarization (open circles), and during the off-depolarization (triangles). Resting potential in darkness was  $-54$  mV.

also increases conductance and has a reversal potential more positive than the dark potential. Since the conductance during the sustained hyperpolarization was always greater than the conductance in darkness it was not possible to assess the changes in excitation which may occur during this part of the response. We assume, however, that there is a light-evoked reduction in excitation and that the resulting conductance decrease is obscured by the larger conductance increase due to the inhibitory input. Thus, it appears that the response may involve changes in excitation and inhibition, which implies that the value of the reversal potential shown in Fig. 2 is the net reversal potential for the hyperpolarizing response and not necessarily that of the inhibitory input itself.

There are a number of reasons to suggest that the light-evoked sustained inhibition is a separate synaptic input rather than simply a maintained component of the transient inhibitory input previously described. First, outward currents associated with transient inhibitory inputs in mudpuppy retinal ganglion cells decay completely within 400–600 msec (Werblin, 1977). Similar transient inhibitory inputs have been described for all classes of tiger salamander ganglion cells and have time-courses of less than 1 sec (Wunk and Werblin, 1979). Finally, we have shown that the transient component of the hyperpolarizing response can be pharmacologically blocked, leaving the sustained component of the response and its associated conductance increase intact (Dvorak *et al.*, 1980).

The presynaptic source of the tonic inhibitory input should be center-depolarizing cells, which include depolarizing bipolar cells and sustained depolarizing amacrine cells. Anatomical investigations and transmitter localization studies tend to favor the amacrine cells (e.g. Famiglietti *et al.*, 1977; Nelson *et al.*, 1978; Bruun and Ehinger, 1974; Voaden, 1976; Marc *et al.*, 1978; Pourcho, 1980), but recent work on cat retina suggests a possible inhibitory role for certain bipolar cells (McGuire *et al.*, 1980).

Dacheux *et al.* (1979) concluded that the light-evoked sustained hyperpolarization of mudpuppy off-center ganglion cells was due entirely to disfacilitation, that is, a reduction in excitation. That conclusion stems from the observation that resistance during illumination was higher than in darkness. In their study, resistance was measured by injecting 0.1 nA depolarizing current pulses through the recording electrode. Since off-center ganglion cells can exhibit a pronounced voltage-dependent conductance increase when they are depolarized beyond the dark potential (see Fig. 2 and also Wunk and Werblin, 1979, for similar data from tiger salamander). To illustrate how this nonlinear property of the membrane can contaminate measurements of synaptically-mediated resistance changes, we have included I–V relations for another cell (Fig. 3) in which strong rectification occurs when the membrane is depolarized as little as 5 mV relative to the dark potential. For this cell, voltage displacements produced by depolarizing currents

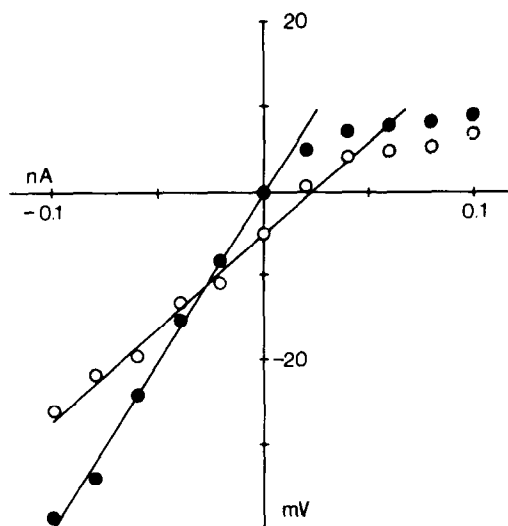


Fig. 3. Current-voltage relations of an off-center ganglion cell measured in darkness (filled circles), and during illumination of the receptive field center (open circles), as in Fig. 2. Stimulus was a 200  $\mu\text{m}$  dia spot, intensity  $-4.6$  log units. Resting potential in darkness was  $-59$  mV.

pulses would indicate a higher resistance in light than in darkness, even though measurements made with hyperpolarizing currents (i.e. over the linear range of the I–V relations) clearly show that resistance is much lower in light than in darkness.

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