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Research Note

An Intensity-Dependent Biphasic Neuron in Mudpuppy Retina

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Intracellular recordings in dark-adapted mudpuppy retinas have revealed a type of infrequently encountered cell with unusual response properties. These cells may be a subclass of horizontal cell since they are encountered at the same depth as horizontal cells and have large receptive fields and response amplitudes. However, they differ from typical horizontal cells in that they are depolarized by low intensity illumination and hyperpolarized by higher intensity illumination at all wavelengths. Both types of responses appear to be driven mainly by 572 nm cones. Both the depolarizing and hyperpolarizing responses were unaffected by APB, indicating that they are not mediated by on-center bipolar cells.

Retina Amphibian Mudpuppy Neuron Cone-driven Biphasic response

INTRODUCTION

In amphibian retinas, second-order neurons are driven by both rods and cones (Fain, 1975; Hassin & Witkovsky, 1983; Witkovsky & Stone, 1987; Wu, 1987). The responses of amphibian horizontal cells are generally monophasic and hyperpolarizing at all wavelengths (L-type), although C-type responses, which are depolarizing to long wavelengths and hyperpolarizing to short wavelengths, have been recorded rarely (Fain, 1975). We have found in mudpuppy retina a previously undescribed type of cell whose responses to brief, bright flashes of light resemble those of horizontal cells. However, they have several unusual properties which distinguish them from typical amphibian horizontal cells: (1) they are depolarized by low intensity illumination and hyperpolarized by higher intensity illumination at all wavelengths; (2) they generate a hyperpolarizing off-response at the termination of the light stimulus; and (3) all components of the response appear to be driven mainly, if not entirely, by 572 nm cones.

METHODS

Intracellular responses were recorded from cells in the eyecup preparation of the mudpuppy *Necturus maculosus*. The preparation, electrical recording and optical stimulation are described in detail elsewhere (Dong & McReynolds, 1991). Briefly, eyecups were prepared under room illumination and superfused with amphibian Ringer solution flowing at approx. 1 ml/min. In some experiments 50 μM 2-amino-4-phosphonobutyric acid (APB) (Sigma) was added to the Ringer solution. After mounting in the recording chamber the preparation was dark adapted for more than 1 hr before beginning an experiment. Intracellular recordings were made with conventional electronics and 4 M potassium acetate-filled micropipettes having resistances of 200–400 MΩ. Light stimuli were from a tungsten–halogen source; the intensity and wavelength were controlled by neutral density and interference filters. Irradiances were measured at the plane of the retina with a calibrated photodiode (UDT-555D).

RESULTS

Figure 1 shows responses of a cell to 100 msec flashes of diffuse light of 500 and 620 nm. The upper row shows responses to a 500 nm flash at two different intensities. A dim flash (left) produced a response that was mainly depolarizing, while a flash 0.8 log unit brighter (right) produced a hyperpolarizing response. The lower row shows responses to 620 nm flashes that were each 0.2 log unit dimmer than the 500 nm flashes above. The weaker 620 nm flash (left) produced a depolarizing response whose amplitude was about equal to that of the depolarizing 500 nm response above. An exact match was not possible because in most cases the stimulus intensity could be adjusted only in 0.2 log unit steps. An 0.8 log unit brighter 620 nm flash (right) produced a hyperpolarizing response. For both the depolarizing and hyperpolarizing responses, the response amplitudes and waveforms were matched when the 500 nm flash was 0.2 log unit brighter than the 620 nm flash. This difference in intensity (indicated by the open circles in Fig. 4) corresponds reasonably well with the relative effectiveness of these two wavelengths for the cone photopigment in mudpuppy (thick curve), which has a λ_{max} of 572 nm (Brown, Gibbons & Wald, 1963; Fain & Dowling, 1973).

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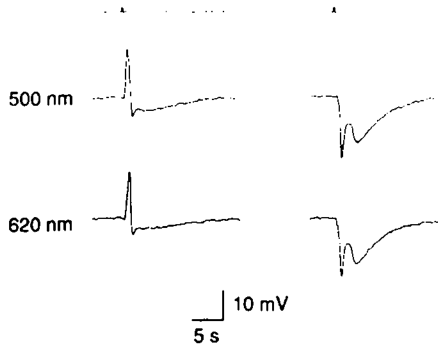

FIGURE 1. Dependence of response polarity on light intensity. Upward deflection of horizontal line above responses indicates light stimulus. Upper row, responses to 500 nm light at intensities of 10.3 (left) and 11.1 (right) log quanta · cm^{-2} · sec^{-1}. Lower row, responses to 620 nm light at intensities of 10.1 (left) and 10.9 (right) log quanta · cm^{-2} · sec^{-1}. All light stimuli were full-field, 100 msec duration.

Note that both the depolarizing and hyperpolarizing 500 nm responses in Fig. 1 were slightly larger than the corresponding 620 nm responses in the lower trace; this means that the responses would be better matched if the intensity of the 500 nm flash was <0.2 log unit brighter than the 620 nm flash, which would result in an even better fit of the data to the cone absorbance spectrum. In contrast, the absorbance spectrum for the rod photopigment (thin curve) predicts that rod-driven responses would only be matched if the 500 nm flash was about 1.1 log unit *weaker* than the 620 nm flash. The results thus indicate that both the depolarizing and hyperpolarizing responses are driven mainly by cones. Both the depolarizing and hyperpolarizing responses show a hyperpolarizing deflection at the end of the response; the use of longer duration stimuli (see below) reveals that this deflection is an off-response.

Figure 2 shows responses from another cell to stimuli of longer duration. The upper row shows responses to 440 nm stimuli of different intensities. The weakest

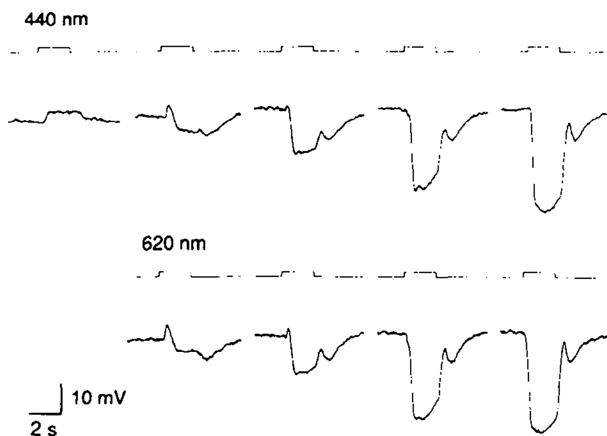

FIGURE 2. Responses to steps of light at different wavelengths and intensities. Upper row, responses to 440 nm light at intensities of (left to right) 8.2, 9.8, 10.4, 10.8 and 11.2 log quanta · cm^{-2} · sec^{-1}. Lower row, responses to 620 nm light at intensities of (left to right) 9.3, 9.9, 10.3 and 10.7 log quanta · cm^{-2} · sec^{-1}. The 620 nm stimulus could not be attenuated to <9.3 log quanta · cm^{-2} · sec^{-1} with the neutral density filters available. All light stimuli were full-field, 2 sec duration.

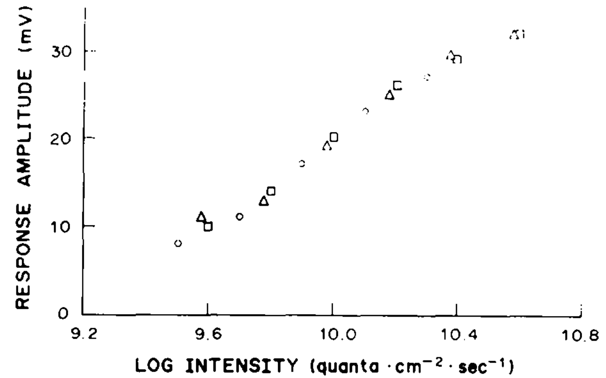

FIGURE 3. Response–intensity functions at different wavelengths. Data are from cell shown in Fig. 2. All light stimuli were full-field, 2 sec duration. Response amplitudes were measured from peak of the initial depolarization, if present, to the potential just before the end of the stimulus, and thus may represent varying mixtures of hyperpolarizing and depolarizing components. Wavelengths of stimuli were 440 nm (triangles), 560 nm (circles) and 620 nm (squares). The abscissa indicates the intensity of the 560 nm stimulus in log quanta · cm^{-2} · sec^{-1}. The data points for the 440 nm stimulus have been shifted to the left by 0.56 log units, and those for the 620 nm stimulus have been shifted to the left by 0.10 log units, to show that the response–intensity functions for all three wavelengths have the same shape. Thus, the intensities for the 440 nm stimuli are actually 0.56 log units greater than indicated by the x-axis scale, and those for the 620 nm stimulus are actually 0.10 log units greater than indicated by the x-axis scale.

stimulus (far left) shows that the depolarizing response was sustained when the intensity was below threshold for the hyperpolarizing component; at higher intensities the hyperpolarizing component, which has a longer latency, appears to cut off the depolarizing response. The lower

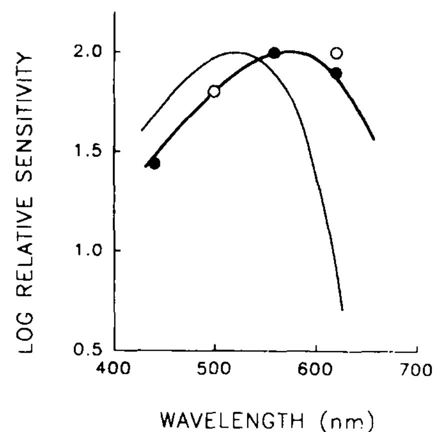

FIGURE 4. Spectral sensitivities of depolarizing and hyperpolarizing responses. Symbols indicate the relative intensities of light needed to produce both hyperpolarizing and depolarizing responses of equal amplitude. Open circles are from the cell in Fig. 1; for both the depolarizing and hyperpolarizing responses, equal response amplitudes were obtained when the 500 nm stimulus was 0.8 log units dimmer than the 620 nm stimulus. Solid circles are from the cell in Fig. 2; the relative sensitivities of the responses of this cell to 440, 500 and 620 nm stimuli were taken from the lateral shifts needed to superimpose the V-log I curves in Fig. 3; the hyperpolarizing and depolarizing responses have the same relative sensitivities to these wavelengths, since stimuli which produced matched hyperpolarizing components also produced matching depolarizing components. Curves show relative absorbance spectra for mudpuppy cones (thick curve) and rods (thin curve). The curves were calculated from a Dartnall nomogram using $\lambda_{max} = 572$ nm for cones and 525 nm for rods (see also Fain & Dowling, 1973).

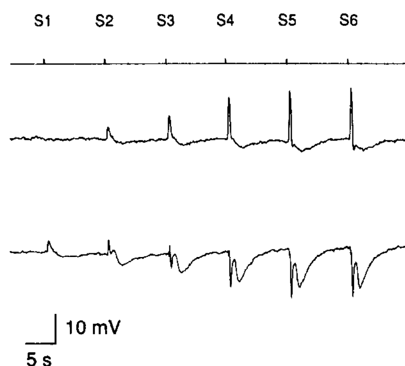

FIGURE 5. Responses to spots of increasing diameter. Upper line is stimulus monitor. All light stimuli were 620 nm, 100 msec duration. Stimuli marked S1–S6 are concentric spots of 95, 245, 420, 670, 1100 and 1700 μm dia, respectively. Intensity was 9.9 log quanta $\cdot$ cm^{-2} $\cdot$ sec^{-1} in upper trace and 10.9 log quanta $\cdot$ cm^{-2} $\cdot$ sec^{-1} in lower trace.

row shows responses to 620 nm stimuli which were each 0.5 log unit dimmer than the corresponding 440 nm stimulus. Both the depolarizing and hyperpolarizing components of the 440 and 620 nm responses were matched in amplitude. The 0.5 log unit difference in intensity corresponds well with the relative effectiveness of these two wavelengths for the 572 nm cone pigment (see below). Unfortunately, because of the greater energy of the light source at 620 nm and the lack of additional neutral density filters, the irradiance of the 620 nm stimulus could not be attenuated to a value 0.5 log units less than the weakest (leftmost) 440 nm stimulus, so that this response was not obtained.

The longer stimuli in Fig. 2 also reveal that the hyperpolarizing deflection seen at the end of the response was an off-response. It is likely that this response component is also due mainly to cone input, since the cone-matched flashes of different wavelengths produced off-responses of the same amplitude and waveform; if the off-responses were rod-driven to a significant extent the 440 nm flash should have produced a larger off-response than the 620 nm flash, since the former was 1.1 log unit more effective for rods than the latter. Furthermore, no off-responses were elicited by very weak 440 nm stimuli, which should have been more effective for rods than for cones.

Figure 3 plots the response amplitude vs stimulus intensity at three different wavelengths: 440 nm (triangles), 560 nm (circles) and 620 nm (squares). Response amplitudes were measured as the difference between the initial depolarizing peak, if present, to the membrane potential just before the end of the light stimulus. The measured values therefore reflect a mixture of the depolarizing and hyperpolarizing components, and the relative contributions of the two may be different at different intensities. The unusual shape of the response–intensity curve may be due to a greater relative contribution of the depolarizing component at low intensities. Nevertheless, these measurements can provide useful information. The response–intensity curves for the three wavelengths were parallel, but at different positions on the intensity axis. In this plot the responses for the 440 and 620 nm stimuli were shifted on the intensity axis until they were superimposed on those of the 560 nm stimulus; the fact that this can be done suggests that the responses were driven by a single type of photoreceptor. The x-axis scale shows the actual intensities for the 560 nm data, which were not shifted. In order to superimpose the response–intensity functions the data points for the 620 nm responses were shifted to the left by 0.10 log unit, and those for the 440 nm response were shifted to the left by 0.56 log units. Thus, the sensitivity of the response at 560 nm is 0.10 log unit greater than at 620 nm and 0.56 log unit greater than at 440 nm. These amounts, indicated by the solid circles in Fig. 4, were very close to the differences (0.12 for 620 nm and 0.53 for 440 nm) predicted from the relative absorbance spectrum for the mudpuppy cone pigment (thick curve). The data do not fit the relative absorbance spectrum for rods (thin curve), which predicts that the sensitivity of rods at 560 nm would be 1.05 log units greater than at 620 nm and 0.23 log unit greater than at 440 nm. The fact that the lateral shifts of the data points required for superimposing the response–intensity functions were the same at all intensities, even though the relative contribution of hyperpolarizing and depolarizing components may have varied with intensity, suggests that both the depolarizing and hyperpolarizing components of the response are mainly due to input from cones.

These cells had large receptive fields. Figure 5 shows responses from another cell to 620 nm spots of increasing diameter (S1–S6) centered in the receptive field. The two rows show responses to the same patterns at two different intensities. At the lower intensity (upper row) the smallest spot was below threshold, but for larger spots the response was depolarizing and increased in amplitude with increasing stimulus diameter. The lower row shows responses to the same series of spots when the intensity was increased by 1 log unit. At this brighter intensity the smallest spot produced a depolarizing response, which changed to a hyperpolarizing response as the stimulus diameter was increased. This may have been due in part to an increase in effective intensity at the receptive field center resulting from increased light scattering into the center as the spot was made larger; in the upper trace, where the intensity was 1 log unit less, light scattering may not have increased the effective intensity enough to produce hyperpolarizing responses. However, the fact that the transition from depolarizing to hyperpolarizing response waveforms with increasing stimulus diameter was quite different in the two traces suggests that these cells may also have a complicated receptive field organization. This could not be further studied due to the failure to record from more cells of this type.

To test the possibility that we were recording from some unusual type of on-center bipolar cell, responses were also recorded in the presence of APB, which eliminates the light responses of on-center bipolar cells (Slaughter & Miller, 1981). In the presence of 50 μM APB, which is enough to completely block the responses

of on-center bipolar cells under our experimental conditions, neither the depolarizing or the hyperpolarizing component of the response was changed (data not shown), indicating that these cells are not on-center bipolar cells. This result also suggests that neither the depolarizing or the hyperpolarizing components of the response is mediated through on-center bipolar cells.

DISCUSSION

The net responses of these unusual cells may be either depolarizing or hyperpolarizing, depending on the intensity of the light stimulus. However, both types of responses appeared to be generated mainly by input from 572 nm cones, since stimuli of different wavelengths which were matched for this cone produced responses that were identical in both amplitude and waveform. The preparations were well dark adapted, but there was no evidence of rod-mediated input; in contrast, rod input could easily be seen in typical mudpuppy horizontal cells in the same retinas under these conditions. Thus, for a given size of the light stimulus the relative proportion of depolarizing and hyperpolarizing components of the response appear to be solely a function of light intensity. Although we cannot rule out the possibility that these cells also receive some rod input, the biphasic nature of the responses seen under our conditions were not due to rod vs cone input. It is unlikely that these unusual responses are some kind of field potential because of their very large size (up to 40 mV) and the fact that light stimuli of these intensities did not produce any detectable responses either just before penetration of the cell or after losing the cell.

The response characteristics of these cells are unlike those of any other identified retinal cells. Their resistance to APB indicates that they are not on-center bipolar cells, and it is unlikely that these cells are a type of photoreceptor or off-center bipolar cell since their responses to small, centered test spots are depolarizing; furthermore, the hyperpolarizing responses to bright stimuli are much larger than ever reported in those cell types. The absence of any action potentials, even when first penetrated, the large amplitude of the hyperpolarizing resposne, and the depth in the retina make it unlikely that these are a type of amacrine or ganglion cell. The responses of interplexiform cells have not been sufficiently well documented to rule them out. Although these cells were encountered at the same depth in the retina as horizontal cells, the lack of rod input at stimulus intensities which normally produce rod responses, and the fact that they were depolarized by dim flashes and hyperpolarized by brighter flashes indicate that they are not conventional horizontal cells. Only one example of a biphasic horizontal cell has been reported in mudpuppy (Fain, 1975), but that cell, unlike the cells described here, was a chromaticity type in which the depolarizing and hyperpolarizing components were wavelength-specific rather than intensity-specific. Due to the extremely low probability of recording from the cells described here (only five examples were encountered in more than four years) and the impracticality of using dye-filled electrodes in all experiments, it was not considered worthwhile to attempt to stain them with dye injection.

Whatever the identity of these cells, it is of interest to briefly consider how full-field responses driven by a single class of photoreceptor may change polarity with different light intensities. For simplicity, we will assume that these cells are second-order neurons and that the transmitter released by photoreceptors is glutamate. One possibility is that the depolarizing and hyperpolarizing components are mediated by different synaptic pathways with different latencies and sensitivities. The fact that no components of the response were affected by APB indicates that on-center bipolar cells are not involved in the circuitry, however. An alternate possibility is that the same photoreceptor transmitter substance acts at two types of receptor sites which have different sensitivities and control different ionic channels. For example, this could occur if these neurons had high-affinity glutamate receptors whose activation leads to depolarization, and low-affinity glutamate receptors whose activation causes hyperpolarization. In the dark, when glutamate release is high, both types of receptors would be activated and the cell would be depolarized, but less so than if only the high-affinity receptor were present. A weak light flash would cause a slight decrease in transmitter concentration, which would mainly affect the activation of the low-affinity glutamate receptor and produce a depolarizing response. A bright flash would cause a net hyperpolarizing response by reducing the activation of both types of receptors.

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