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Electrical coupling between rods and cones in the tiger salamander retina

(spectral sensitivity/response waveform/Purkinje shift/dark and light adaptation)

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ABSTRACT **Electrical coupling between rods and cones was studied in the salamander (*Ambystoma tigrinum*) retina by measuring the light responses and spectral sensitivities of rods and cones and by measuring the voltage responses from a rod to current pulses injected into a cone. A population of 10–20% of the photoreceptors exhibited a mixed-response waveform of the rod and the cone under dark-adapted conditions, and a response waveform closely resembled that of a cone in the presence of background illumination. Lucifer yellow injection revealed that these cells are morphologically identical to rods, and thus they are named rod$_c$s. Dark-adapted rod$_c$s exhibited a rod-like spectral sensitivity with a peak at ≈520 nm that shifted to a cone-like spectral sensitivity with a peak at ≈620 nm in response to background light (Purkinje shift). The voltage response of a rod$_c$ to a −1-nA current step injected into an adjacent cone is ≈3.6 times larger than that of a rod to the same current step. These results indicate that there is a population of rods (rod$_c$s) in the tiger salamander retina that is strongly coupled to the cones and that these cells allow significant mixture of rod and cone signals at the photoreceptor level.**

In the vertebrate retina, photoreceptors are electrically coupled to one another, and this coupling is mediated by low-resistance gap junctions that allow current to flow directly between cell interiors (1–7). It is generally believed that electrical coupling occurs primarily between photoreceptors of the same type, and thus electrical signals can be averaged over a number of photoreceptors, smoothing fluctuation introduced by the quantum nature of light (4, 8). Photoreceptors of various types—rods and cones, for example—are weakly coupled to each other (5, 7). Since rods and cones operate under different luminance conditions and exhibit different response waveforms (9, 10), it is not clear what function the coupling between these two photoreceptors serves.

In this report, we present evidence suggesting that there is a population of rods (rod$_c$s) in the tiger salamander retina that is strongly coupled to the cones, whereas the rest of the rods are weakly coupled to the cones. The following three independent measurements were made under dark- and light-adapted conditions to examine the difference between the rod–cone interactions and the rod$_c$–cone interactions: (*i*) waveform of the light responses, (*ii*) spectral sensitivities, and (*iii*) voltage response of a rod (or rod$_c$) to current injections into an adjacent cone. Results obtained suggest that significant mixture of rod and cone signals occurs at the photoreceptor level in the rod$_c$s, that rod$_c$s behave as hybrid photoreceptors under dark-adapted conditions, and that they behave like cones in the presence of background illumination.

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MATERIALS AND METHODS

The Flat-Mounted Isolated Retina. Larval tiger salamanders (*Ambystoma tigrinum*) purchased from the Lowrance Waterdog Farm (Tulsa, OK) were used in this study. Prior to an experiment, the animal was dark-adapted overnight and then decapitated under infrared illumination. The eyes were enucleated and hemisected. A piece of the posterior half of the eyecup was inverted over a hole in a piece of Millipore filter (HAO; pore size, 0.45 μm) secured in the superfusion chamber. The sclera and the pigment epithelium were removed from the retina. Oxygenated Ringer's solution (108 mM NaCl/2.5 mM KCl/1.2 mM $MgCl_2$/2 mM $CaCl_2$/5 mM Hepes, pH 7.7) was added to the superfusion chamber, so that the retina was immersed totally under solution. The entire procedure was done under infrared illumination with a dual-unit Fine-R-Scope (FJW Industry, Mount Prospect, IL). The retina (photoreceptor-side up) was viewed with a Zeiss water-immersion 40× objective lens modified for the Hoffman modulation contrast optics (Hoffman Modulation Optics, Greenvale, NY). The working distance of the objective lens was ≈1.6 mm, thus two electrodes could be placed into retinal cells at an angle of 15° from the horizontal. During the experiment, photoreceptors as well as the two electrodes were clearly observed on the screen of a TV monitor connected to the infrared image converter (model 4415; COHU, Palo Alto, CA) attached to the microscope.

Rods and cones could be identified by their morphology in the flat-mounted retinas (plate 4 in ref. 11) before electrode penetration, and their identities were confirmed subsequently by the waveform of their light responses. Rods and rod$_c$s were morphologically identical in the flat-mounted retinas, and thus the distinction between these two cell types was made by the waveform and spectral sensitivity measured after electrode penetration. The morphology of rod$_c$s in transverse retinal sections was established by Lucifer yellow injection (see Fig. 2).

Recording and Stimulation. Intracellular recording and current injection were made with micropipettes drawn with a modified Livingston puller with Omega Dot tubing (1.0-mm o.d. and 0.5-mm i.d.). The micropipettes were filled with 2 M potassium acetate and had tip resistances, measured in Ringer's solution, of 100–600 MΩ.

The membrane potential of photoreceptors was recorded as the potential difference between the intracellular recording electrode and the bath electrode. Two intracellular electrodes were inserted simultaneously into two photoreceptors to measure the strength of electrical coupling: one for passing constant current pulses and the other for recording the voltage responses. Photoreceptors in the flat-mounted retinas were impaled under visual control, and the impalement was facilitated by adjusting the negative capacitance in the electrode headstage. Voltage and current traces were monitored with an oscilloscope (model 5500A; Tektronix, Beaverton, OR) and stored in magnetic tapes (Racal 7DS).

Lucifer Yellow Staining. Intracellular micropipettes were backfilled with Lucifer yellow [kindly supplied by Walter Stewart (National Institutes of Health); 4% (wt/vol) in 1 M LiCl). Steps of −10-nA (2 Hz) current were applied for 2–5 min for dye injection. The retina was fixed for 1 hr in 4% (wt/vol) paraformaldehyde/0.1% glutaraldehyde/100 mM sodium phosphate (pH 7.4) and then embedded in 4% (wt/vol) agarose. The retina was sectioned (50 μm thick) with a vibratome (Pelco 1000, Ted-Pella, Tustin, CA).

Solutions. Preparations were maintained at room temperature (20–23°C) in an oxygenated Ringer's solution.

Light Source. The preparation was stimulated with a dual-beam photostimulator. Two independent light beams, whose intensity and wavelength could be adjusted by neutral-density filters and interference filters, were provided by quartz halogen sources. The light was transmitted to the preparation by way of the epiilluminator and the objective lens of the microscope, and the spot diameter on the retina could be adjusted by a diaphragm in the epiilluminator. In most experiments described, large-field illumination (600–800 μm in diameter) was used. The light sources were calibrated with a radiometric detector (United Detector Technology, Santa Monica, CA).

RESULTS

Fig. 1 shows the light responses of a rod, a cone, and a rod_c under dark-adapted conditions (*A*) and in the presence of background illumination (*B*). Rod_c was morphologically identical to a rod (Fig. 2), but it exhibited a mixed waveform of rod and cone responses to a bright light step under dark-adapted conditions (Fig. 1*A*): it repolarized immediately after the termination of the light step (like cones), and

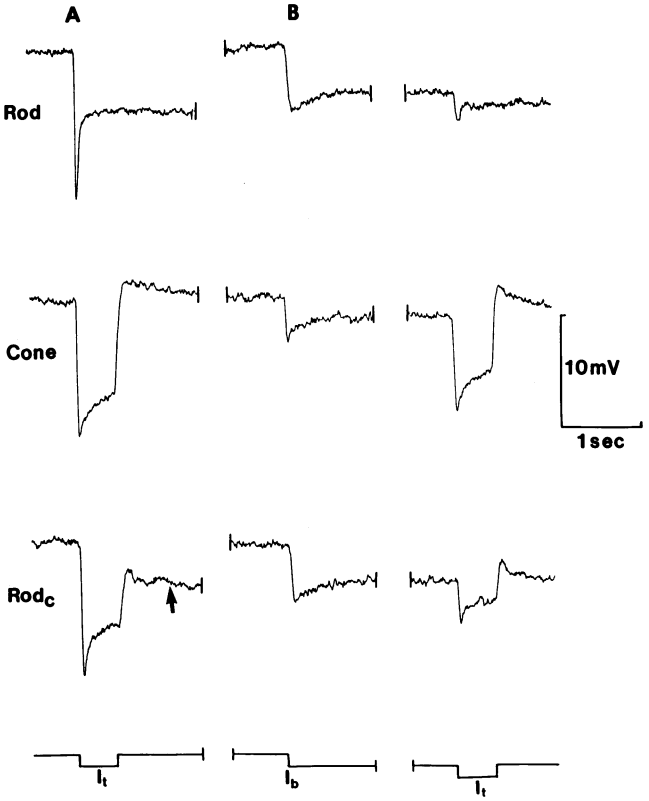

FIG. 1. Voltage responses of a rod, a cone, and a rod_c to a bright 520-nm light step (1.47×10^7 photons per μm^2/sec) under dark-adapted conditions (*A*) and in the presence of background illumination (white light, 0.043 $\mu W/\mu m^2$) (*B*). The dark resting potentials of the rod, the cone, and the rod_c were −44 mV, −41 mV, and −42 mV, respectively.

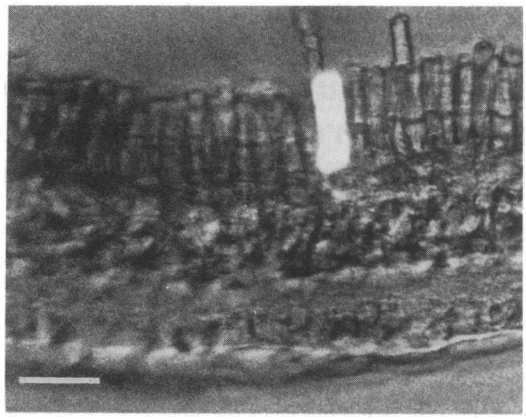

FIG. 2. Lucifer yellow-filled rod_c in the tiger salamander retina. This cell exhibited light responses and spectral sensitivity of the rod_c (see Figs. 1 and 3) before dye injection. Lucifer yellow was never observed in photoreceptors adjacent to the injected cell. (Bar = 20 μm.)

it gave a long voltage tail (arrow) afterward (like rods). The waveform of the rod_c response to dim light steps (data not shown) resembled the waveform of the rod. Among 205 rods recorded, 24 exhibited the response waveform of the rod_c, and the rest exhibited the waveform of the rod. Fig. 1*B* shows the voltage responses of the same three cells to the same bright test light step after a steady background light was introduced to the retina. The rod response was suppressed almost completely, whereas the cone response was only slightly reduced. The response of rod_c under this condition closely resembled the waveform of the cone response but with a smaller amplitude. These results indicate that the light response of rod_c was probably a mixture of the rod and cone responses under dark-adapted conditions and became a cone-dominant response after the rod responses were suppressed by background illumination.

Fig. 3 shows the spectral sensitivity curves for a rod, a cone, and a rod_c under dark-adapted conditions and in the presence of background illumination. Under dark-adapted conditions, the maximum spectral sensitivities of the rod and the cone were ≈520 nm and 620 nm, respectively. The spectral sensitivity curve of rod_c determined by a small criterion response (2.5 mV) under dark-adapted conditions resembled that of the rod (peak at 520 nm), except that the rod_c curve had a more shallow slope at the long wavelength side. The spectral sensitivity curve of rod_c determined by larger criterion responses (data not shown) under dark-adapted conditions gave a progressively shallower slope at the long wavelength side, indicating for brighter test light steps, cone signals became progressively larger in rod_c. These observations are consistent with the hypothesis that the light response of rod_c is a mixture of the rod and cone responses under dark-adapted conditions. In the presence of background illumination, the spectral sensitivities of the rod and the cone did not change significantly from their dark-adapted values (Fig. 3 *A* and *B*). The spectral sensitivity of rod_c measured in the presence of background illumination, on the other hand, was very different from that measured under dark-adapted conditions: instead of a rod-like spectral sensitivity curve, rod_c exhibited a spectral sensitivity that resembled that of the cone with a peak of ≈620 nm (Fig. 3*C*). This demonstrates that the intracellular responses of the dark-adapted rod_c can exhibit Purkinje shift in response to steady background illumination.

Results described above indicate that rod_c exhibits a mixed response of rods and cones under dark-adapted conditions while having a rod-like morphology, and it is likely that these cells are rods strongly coupled with the

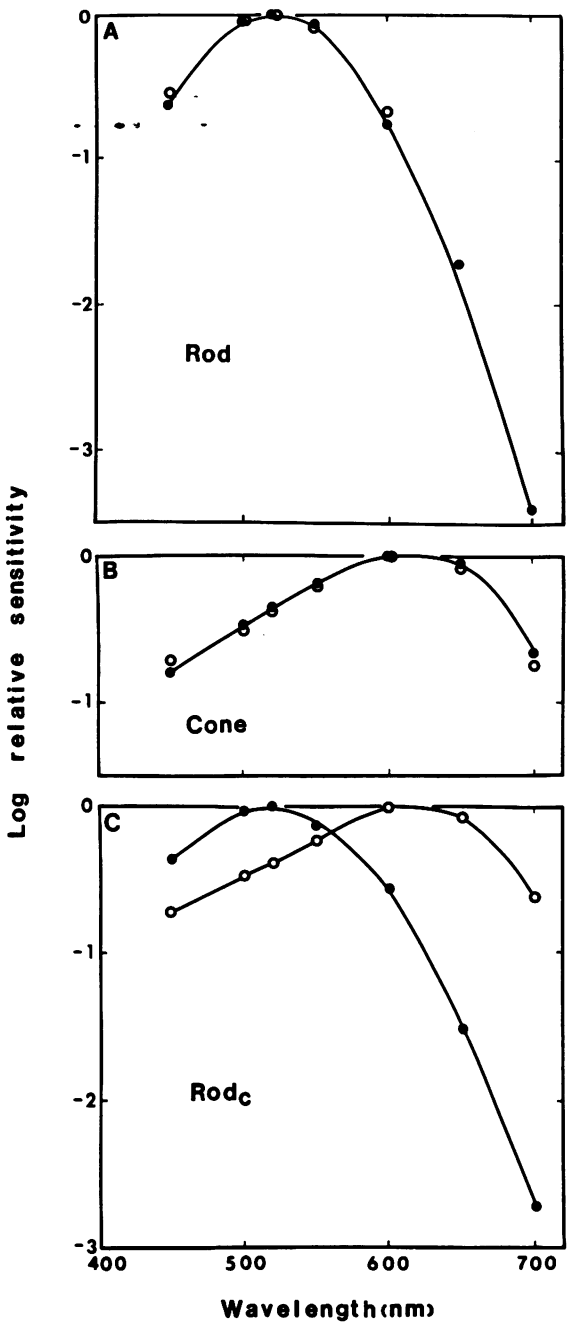

FIG. 3. Normalized spectral sensitivity curves of a rod (*A*), a cone (*B*), and a rod$_c$ (*C*). Solid circles represent data obtained under dark-adapted conditions, and open circles represent data obtained in the presence of background illumination (white light, 0.043 $\mu W/\mu m^2$). Criterion response for all data points was 2.5 mV. Under dark-adapted conditions, the maximum spectral sensitivity of the rod was ≈520 nm and that of the cone was ≈620 nm. Dark-adapted rod$_c$ showed a spectral sensitivity very close to that of the rod, except it had shallower slope at the long wavelength side. In the presence of background illumination, data points from the rod and the cone (open circles) were almost superimposable to the values obtained under dark-adapted conditions. Rod$_c$ under background illumination gave a spectral sensitivity curve different from the one obtained under dark-adapted conditions: it changed from a rod-like spectral sensitivity to a cone-like spectral sensitivity, with the peak value shifted from 520 nm to 620 nm (Purkinje shift).

cones. To test this hypothesis, two electrodes were placed into a cone and a rod (or a rod$_c$) that were next to each other under visual control in the flat-mounted isolated retina. Current pulses were passed into the cone while the voltage responses were recorded from the rod (or rod$_c$). Rod$_c$s were morphologically indistinguishable from other rods (Fig. 2), and they were identified by their characteristic waveform of the light responses (Fig. 1*A*). Fig. 4 shows the voltage response of rod$_c$ to a −1-nA current step injected into an adjacent cone is ≈3.6 times larger than that of a rod to the same current step. Reverse current injection elicited responses of the same amplitude in cones (data not shown, but see figure 2 of ref. 10). This suggests that the strength of coupling between rod$_c$s and cones (9.4 ± 2.8 mV response to −1 nA into an adjacent cone, n = 5) is significantly stronger than that between rods and cones (2.8 ± 1.7 mV response to −1-nA current into an adjacent cone, n = 14). The strength of coupling between rod$_c$s and rods (11.9 ± 3.5 mV response to −1-nA current into an adjacent rod, n = 4) is not significantly different from that between two adjacent rods (6). Rod$_c$s were never encountered as adjacent pairs in this retina. It is likely that the strong electrical synapses between rod$_c$s and the cones mediate the mixture of the rod and cone responses in rod$_c$ under dark-adapted conditions. These synapses also enable rod$_c$ to exhibit a cone response in the presence of background illumination when rod responses were suppressed.

DISCUSSION

Experiments described in this article demonstrate that there is a population of rods (rod$_c$s) in the tiger salamander retina that are strongly coupled to the cones. These cells are morphologically indistinguishable from other rods but behave as a hybrid of rods and cones under dark-adapted conditions. In addition to mixing rod and cone signals in second- or higher-order cells, the retina pools rod and cone responses in rod$_c$s. From the current injection experiments, the strength of coupling between rods and cones follows a bimodal distribution, indicating that rods and rod$_c$s are two

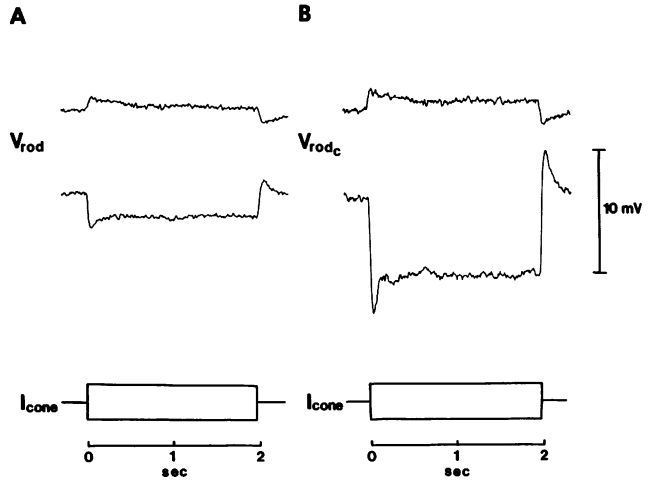

FIG. 4. Voltage responses of a rod (*A*) and a rod$_c$ (*B*) to ±1 nA of current pulses injected into an adjcent cone (I_{cone}). Reverse current injection elicited similar responses in the cones (data not shown, but see figure 2 of ref. 10). The +1-nA current gave smaller responses than the −1-nA current because of the outward rectification of the photoreceptor plasma membrane (6). When given a 1-nA current pulse, rod$_c$ gave a voltage response whose steady-state value was ≈3.6 times larger than that of the rod, indicating the coupling between rod$_c$ and the cone is much stronger than that between the rod and the cone. In fact, the instantaneous response of rod$_c$ shown in *B* was ≈4.2 times larger than that of the rod in *A*. The on-transience of the rod$_c$ response was larger because the cell was more hyperpolarized, and a larger time-dependent inward current was activated (6). The resting potentials of the rod and the cone in *A* were −40 mV and −42 mV, respectively, and the resting potentials of the rod$_c$ and the cone in *B* were −43 mV and −40 mV, respectively.

distinct populations of photoreceptors. Rod_cs account for 10–20% of the total photoreceptors in the tiger salamander retina. If these cells make the same synaptic contacts onto second-order cells as other rods, then 10–20% of the photoreceptor signals arriving at the second-order cells are mixed signals, whereas the other 80–90% are either rod or cone signals. Mixture of rod and cone signals at the photoreceptor level is not limited to the salamander retina. In cats, substantial rod inputs can be recorded from horizontal cells that only contact the cones (12). It is possible that the results described in this article are applicable to some other vertebrate species.

Results in this article also demonstrate that rod_cs show a Purkinje shift in response to background illumination. A Purkinje shift has been traditionally attributed to changes of dominance of rod and cone signals in higher-order retinal cells (13). Here we present evidence that demonstrates that Purkinje shift occurs in a population of photoreceptors, the first-order neuron in the visual system. In addition to changing the spectral sensitivity of rod_cs, background illumination also alters the response waveform of these cells. In the presence of background light, when rod signals were suppressed, rod_cs exhibited a response waveform that closely resembled that of the cones. This results in an abrupt repolarization of the rod_c voltage at the cessation of the light step. For bright light steps, an anode break regenerative potential sometimes can be activated by this abrupt repolarization (14).

In the tiger salamander retina, there is primarily only one type of rod pigment and one type of cone pigment (7), and such organization is disadvantageous for encoding color information. Mixing rod and cone responses in rod_cs through electrical synapses provides a third type of photoreceptor under scotopic or mesopic conditions that has a spectrum different from those of the rod and cone pigments. Under mesopic conditions, for brighter background illumination, the peak spectral sensitivity of rod_c moved progressively from 520 μm to the right, until it reached $\approx$620 nm (photopic condition). Color discrimination is considered a cone-mediated function in most vertebrate species (15–17). Nevertheless, in a retina where only one type of cone is available, rod_cs can serve the function of spectrally distinct cones. This may increase the ability of the tiger salamander retina to distinguish between different colors. Under photopic conditions, when rod signals are suppressed, cone signals can be transmitted to the second-order cells in two ways: either directly through the cone synapses or indirectly through rod_cs presumably by way of rod synapses. It is probably economical, if not advantageous, for this spectrally simple retina to utilize part of the rod pathways for cone signals under the conditions when rod responses are suppressed.

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1. Baylor, D. A., Fuortes, M. G. F. & O'Bryan, P. M. (1971) *J. Physiol.* **214,** 256–294.
2. Baylor, D. A. & Hodgkin, A. L. (1973) *J. Physiol. (London)* **234,** 163–198.
3. Fain, G. L., Gold, G. H. & Dowling, J. E. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **40,** 547–561.
4. Lamb, T. D. & Simon, E. J. (1976) *J. Physiol. (London)* **263,** 257–286.
5. Schwartz, E. A. (1976) *J. Physiol. (London)* **257,** 379–406.
6. Attwell, D. & Wilson, M. (1980) *J. Physiol. (London)* **309,** 287–315.
7. Attwell, D., Wilson, M. & Wu, S. M. (1984) *J. Physiol. (London)* **352,** 703–737.
8. Baylor, D. A. (1978) in *Theoretical Approaches in Neurobiology*, ed. Reichardt, W. E. & Poggio, T. (MIT, Cambridge, MA), pp. 18–27.
9. Schultze, M. (1866) *Arch. Mikrosk. Anat.* **2,** 175.
10. Attwell, D., Werblin, F. S., Wilson, M. & Wu, S. M. (1983) *J. Physiol. (London)* **336,** 313–333.
11. Werblin, F. S. (1978) *J. Physiol. (London)* **280,** 449–470.
12. Nelson, R., Lynn, T., Dickinson-Nelson, A. & Kolb, H. (1985) in *Neurocircuitry of the Retina: A Cajal Memorial*, ed. Gallego, A. & Gouras, M. D. (Elsevier, New York), pp. 109–121.
13. Rushton, W. A. H. (1959) *J. Physiol. (London)* **149,** 327–345.
14. Wu, S. M. (1987) *Vision Res.* **27,** 143–150.
15. Mollon, J. D. (1982) *Annu. Rev. Psychol.* **33,** 41–85.
16. Zrenner, R. (1983) in *Studies of Brain Function*, eds. Braitenberg, V., Barlow, H. B., Bullock, T. H., Florey, E., Grusser, O.-J. & Peters, A. (Springer, Berlin), Vol. 9.
17. Daw, N. W. & Pearlman, A. L. (1970) *J. Physiol. (London)* **211,** 125–137.