Published in final edited form as:

J Comp Neurol. 1998 September 7; 398(4): 529–538.

Dopamine D2 Receptor-Mediated Modulation of Rod-Cone Coupling in the *Xenopus* Retina

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Abstract

We studied the responses of rod photoreceptors that were elicited with light flashes or sinusoidally modulated light by using intracellular recording. Dark-adapted *Xenopus* rod photoreceptors responded to sinusoidally modulated green lights at temporal frequencies between 1 Hz and 4 Hz. In normal Ringer's solution, 57% of the rods tested could follow red lights that were matched for equal rod absorbance to frequencies >5 Hz, indicating an input from red-sensitive cones. Quinpirole (10 μ M), a D2 dopamine agonist, increased rod-cone coupling, whereas spiperone (5 μ M), a selective D2 antagonist, completely suppressed it. D1 dopamine ligands were without effect. Neurobiotin that was injected into single rods diffused into neighboring rods and cones in quinpirole-treated retinas but only diffused into rods in spiperone-treated retinas. A subpopulation of rods (ca. 10% total rods) received a very strong cone input, which quickened the kinetics of their responses to red flashes and greatly increased the bandpass of their responses to sinusoidally modulated light. Based on electron microscopic examination, which showed that rod-rod and cone-cone gap junctions are common, whereas rod-cone junctions are relatively rare, we postulate that cone signals enter the rod network through a minority of rods with strong cone connections, from which the cone signal is further distributed in the rod network. A semiquantitative model of coupling, based on measures of gap-junction size and distribution and estimates of their conductance and open times, provides support for this assumption. The same network would permit rod signals to reach cones.

Keywords

gap junction; photoreceptor; retina; neuromodulation

When rod photoreceptors are fully dark-adapted, they respond reliably to the absorbance of a single quantum of light (Yau et al., 1977). Rods continue to function when they are desensitized by background lights (Fain, 1976) or by prior exposure to light, and, in those circumstances, the effective stimuli for rods can be sufficiently bright to also activate cone photoreceptors, allowing for the possibility of interactions between rod signals and cone signals. One possible

site for rod-cone communication is an electrotonic junction between photoreceptors, whose morphological sign is the gap junction. Gap junctions joining rods and cones have been identified (Raviola and Gilula, 1973), and functional rod-cone coupling in primate retina has been reported (Schneeweis and Schnapf, 1995).

The main experimental question of the present study is whether the conductances of photoreceptor gap junctions are subject to neuromodulation. We focus on the intrinsic retinal neurochemical, dopamine (Haggendal and Malmfors, 1965), which is known to modulate the gap-junctional conductance of retinal second-order neurons (Piccolino et al., 1984) and to augment information flow in cone circuits while suppressing that in rod circuits (Witkovsky and Dearth, 1991). Photoreceptors have dopamine receptors of the D2/D4 subtypes (Cohen et al., 1992; Muresan and Besharse, 1993). In the present study, we found that a D2 dopamine agonist, but not a D1 agonist, increases rod-cone coupling in the *Xenopus* retina, resulting in altered rod light-evoked response kinetics and an increased ability of rods to follow sinusoidally modulated red light at temporal frequencies > 5 Hz. The underlying circuit for cone-to-rod signal transfer appears to depend on a subclass of rods that receives strong cone input. A similar subtype of rod was identified in the salamander retina (Wu and Yang, 1988). We postulate that the rods receiving strong cone input distribute the cone signal to other rods, presumably through rod-rod gap junctions, which are very numerous in amphibian retinas (Gold and Dowling, 1979).

MATERIALS AND METHODS

Male *Xenopus*, 6–8 cm crown-rump length, were obtained from NASCO (Ft. Atkinson, WI) and maintained in an aerated aquarium on a 12 hour-12 hour light-dark cycle, with lights on at 6 AM. Animals were anaesthetized with Tricaine methanesulfonate (20–30 mg in Ringer's solution) injected subcutaneously. Under deep anesthesia, the animals were decapitated and pithed, and the eyes were enucleated. Our animal handling and surgical procedures were approved by the Animal Care Committee of New York University Medical Center. The front half of the eye was removed, and the posterior pole was pinned flat in a wax chamber and superfused with a bicarbonate Ringer's solution, pH 7.4, at 1.5 ml minute⁻¹. The composition of the Ringer's solution (in mM) was NaCl, 100.0; KCl, 2.0; CaCl₂, 1.8; MgCl₂, 1.0; NaHCO₃, 25.0; ascorbate, 0.2; and glucose, 10.0. Intracellular pipettes were filled with 4 M K acetate and had resistances of 150–300 Mohm. The procedures for intracellular recording and data collection were standard and are fully described in Krizaj et al. (1994). Light stimuli were provided by green (567 nm) and red (660 nm) LEDs that were driven by a function generator. The stimuli covered the eye cup with diffuse, even illumination. Light intensity was adjusted with a neutral density wedge. Light intensities were measured by using a photomultiplier that was referenced to a calibrated thermopile and are expressed as log quanta incident cm⁻² s⁻¹.

Dopamine ligands (quinpirole HCl, spiperone, SCH 23390, SKF 38393) and glutamate ligands (quisqualate, kainate, and 6-cyano-7-nitro-quininoxaline-2,3-dione [CNQX]) were obtained from Research Biochemicals International (Natick MA). Quinpirole HCl and SKF 38393 were dissolved directly in Ringer's solution. Spiperone was first dissolved in ethanol or dimethyl sulfoxide and was then diluted 200- to 1,000-fold in Ringer's solution. SCH 23390 was first dissolved in weak acetic acid and was then diluted 1,000-fold in Ringer's solution.

For cell marking, we used 4% (weight/volume) Neurobiotin (Vector Laboratories, Burlingame, CA) in 0.1 M Tris-HCl, pH 7.6. Rods were injected for 4 minutes with 3 Hz sinusoidal current, direct current (DC) biased to be positive, peak current 0.5–0.8 nA. After at least 15 minutes post current injection, the retina was stripped from the eye cup, flattened on filter paper, and fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After several

washes in phosphate-buffered saline (PBS), the retina was treated with 0.5% Triton X-100 in PBS, incubated with a streptavidin-biotinylated horseradish peroxidase complex, then visualized with the standard diaminobenzidine reaction. The retina was flat-mounted in glycerol, and the stained cells were identified and photographed. Subsequently, the retina was freed from the slide, washed in PBS, dehydrated, and embedded in Durcupan (Fluka, Buchs, Switzerland). The portion of the tissue containing the stained cells was sectioned serially at 2 μ m and was lightly counterstained with toluidine blue. Electron microscopy was carried out by using standard methods on retinas fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% buffered OsO₄, and stained overnight en bloc with saturated uranyl acetate in 70% ethanol.

RESULTS

Rod responses to sinusoidally modulated light

We examined rod responses to sinusoidally modulated green (567 nm) or red (660 nm) lights by using intracellular recording. Because prior light history and time of day influence rod performance, the recording conditions were standardized. Frogs were entrained to a 12 hour-12 hour light-dark cycle, with dawn at 6 PM. Eyecups were prepared in room light between noon and 1 PM and were allowed to dark adapt for 2–3 hours, then, rod recordings were obtained between 3 PM and 6 PM. These procedures notwithstanding, we found that rods varied substantially in their operating range, i.e., the position of the response-intensity function along the intensity axis, and in their kinetics. The voltage-intensity functions showed that the quantal flux (567 nm) for half-saturation typically was in the range 9.7–10.2 log quanta incident/cm²/second, indicating that the rods studied were 0.5–1.0 log units less sensitive than fully dark-adapted *Xenopus* rods (Engbretson and Witkovsky, 1978). Rods also showed variability in their responses to sinusoidal red stimuli > 5 Hz. In contrast, the responses to green sinusoids of moderate (i.e., with little effect on cones) intensity were uniform among all rods tested.

In retinas that were superfused with normal Ringer's solution, we distinguished four functional types of rods (Fig. 1), based on an examination of their responses to brief flashes of red or green light or to sinusoidally modulated light stimuli ($n = 92$). It is possible that the functional categories we used to distinguish rod responses are part of a continuum; however, they were found to be convenient for quantifying and categorizing the diversity of rod responses. Type A rods (43% total) showed a progressive decline in peak-to-peak response amplitude as the sinusoidal frequency increased from 1 Hz to 5 Hz. Type B rods (30%) were similar to type A between 1 Hz and 4 Hz, but they showed an increase in response beginning at 6 Hz. Type C rods (17%) were able to follow low-frequency sinusoids with relatively little attenuation compared with types A or B, whereas, for type D rods (11%), there was no decline in response amplitude between 1 Hz and 4 Hz, and these rods followed sinusoids to 20 Hz.

The ability to follow sinusoidal stimuli above 5 Hz was seen only for red stimuli, indicating an input from a red-sensitive photoreceptor. Among the five photoreceptor classes in *Xenopus* retina (two rods, three cones; Witkovsky et al., 1981; Zhang et al., 1994), only one is red sensitive. This red-sensitive cone constitutes 89% of the cone population (Zhang et al., 1994), whereas the principal rod (524 nm pigment; Witkovsky et al., 1981) makes up at least 97% of all rods. Thus, we assume that the higher frequency responses are transmitted into green-sensitive rods from red-sensitive cones.

A bright flash elicits a characteristic response from vertebrate retinal rods, which consists of a transient hyperpolarization that relaxes to a maintained plateau (Baylor et al., 1984). We observed that increased cone input to rods was associated with altered kinetics of the rod's response to a brief test flash, as shown on the right in Figure 1.

For the type A rod, which has no discernible cone input, red and green lights matched for equal rod absorbance elicited identical flash responses. Thus, type A rods illustrate the principle of univariance (Rushton et al., 1973), whereby equal quantum catch is associated with identical response amplitude and kinetics, irrespective of the wavelength of stimulating light. For the type C rod, however, red and green lights that were matched for equal rod absorbance evoked different rod responses; specifically, the initial transient (Fig. 1, arrow) was much greater in response to the red flash. This wavelength-dependent difference indicates an input from another red-sensitive cell type. The shape of the transient included a sharp depolarization at light off, which is not a characteristic of the rod's light response but is typical of a cone's response to light when the stimulus duration exceeds about 100 msec (Normann and Perlman, 1979).

Figure 2 illustrates that there is a positive correlation between the ability to follow higher frequency sinusoids and the fractional size of the rod transient. Thus, for a type A rod that showed no ability to follow frequencies > 5 Hz, the transient was $0.41 V_{\max}$, increasing to $0.70 V_{\max}$ for a type D rod, which could follow sinusoidal stimuli to 20 Hz.

In principle, rod-cone communication could occur through chemically mediated synapses, electrotonic junctions, or both. Because both rods and cones release glutamate (Ayoub and Copenhagen, 1991; Schmitz and Witkovsky, 1996), we superfused the retina with the glutamate receptor agonists, quisqualate (10 μ M) or kainate (10 μ M), while monitoring rod responses to sinusoids (not illustrated). Neither of these agents affected the rod response, nor did CNQX (100 μ M), which is a blocker of α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors (Honore, 1991).

Neurobiotin injections into rods

To test for electrotonic communication, we injected rods with neurobiotin, which permeates gap junctions (Vaney, 1991). Injections were made into retinas that were superfused with normal Ringer's solution ($n = 21$), with the D2 dopamine antagonist spiperone (5 μ M; $n = 10$), or with the D2 dopamine agonist quinpirole (10 μ M; $n = 12$). Typically, only one injection (but never more than two injections) was made per retina. Photomicrographs of marked cells are shown in Figure 3, and the numbers of rods/cones stained are provided in Table 1. When they were viewed in retinal wholemounts, the stained cells typically overlapped, so that it was not always possible to identify the number of stained photoreceptors and what fraction was rods or cones. Thus, each test retina was embedded in plastic, 2- μ m-thick, serial sections were obtained through the stained site, and the marked cells were identified as rods or cones.

The results summarized in Table 1 document that, irrespective of the bathing medium, neurobiotin passed from the injected cell into a group of neighboring rods. In control Ringer's solution, 7 of 21 injection sites contained a single cone, and, in one other injection, two cones were marked. None of the marks obtained in spiperone-treated Ringer's solution contained a cone, whereas 8 of 12 injections in quinpirole-treated Ringer's solution contained one cone, two injection sites contained two cones, and only two injection sites lacked a stained cone. The number of stained rods was not statistically different between control and spiperone-Ringer's solution, but it was slightly smaller in quinpirole-Ringer's solution. These data indicate that rods and cones communicate through gap junctions and that spiperone cuts off rod-cone coupling, whereas quinpirole enhances it.

Effects of D1/D2 dopamine ligands on rod responses to sinusoids

Next, we assessed the effects of quinpirole (10 μ M) and spiperone (5 μ M) as well as the D1 agonist, SKF 38393 (25 μ M), and the D1 antagonist, SCH 23390 (10 μ M), on rod responses to sinusoidally modulated red stimuli. The retina was superfused with the test drug for at least 15 minutes before testing the rods, and the results are shown in Figure 4. The main finding is

that the D2 agonist altered the distribution of rod subtypes toward a greater proportion that was able to follow relatively high frequencies, whereas the D2 antagonist completely suppressed the ability to follow red sinusoids > 5 Hz. In fact, spiperone-exposed rods showed no difference in responses elicited by red or green sinusoids, indicating a complete loss of cone input. The D1 agonist, SKF 38393, had no marked effect on rod responses to sinusoids, nor did the D1 antagonist, SCH 23390 (not illustrated)

Characterization of type D rods

The first question was whether the responding cells truly were rods. We succeeded in injecting neurobiotin into three cells, identified as type D on the basis of their response to sinusoids (illustrated in Fig. 1). All three were rods of normal appearance. An unusual feature of the type D rod, however, was brought out by comparing responses to 567 nm and 660 nm flashes at different intensities. The upper records in Figure 5 illustrate that, even near threshold, the response elicited by the red flash has much faster kinetics than that evoked by a green flash. When the two flashes were matched to elicit equivalent plateau components (Fig. 5, middle records), the initial transient was much faster to red than to green light. For very bright stimuli (Fig. 5, lower records), the two transient responses merge to produce a very large initial transient. Thus, the response kinetics indicate that type D rods receive a very powerful red cone input, which greatly alters the kinetics of the cells' light-evoked responses. The fast kinetics of type D rods relative to those of rods with a small to absent cone input are particularly noticeable in responses to sinusoidal red stimuli (Fig. 1, lower left).

Electron microscopic characterization of photoreceptor gap junctions

Given the variability within the rod population with respect to the degree of cone input, two models for communication seemed possible. At one extreme, every rod would be joined by a variable number of gap junctions to cones, allowing for different degrees of rod-cone conductance. At the other extreme, only type D rods would receive cone input, which would be communicated to ordinary rods through rod-rod gap junctions.

We explored this question by studying the distribution, relative numbers and dimensions of gap junctions between photoreceptors. We paid particular attention to the photoreceptor fins, which are gear-like extensions from the inner segment, for it is in this region that numerous photoreceptor gap junctions have been found in the toad (*Bufo*) retina (Fain et al., 1976). In the *Xenopus* retina, however, although both rods and cones have fins (Fig. 6a–c), which come into close proximity, this is not a site of gap-junction formation. Instead gap junctions typically are formed by direct apposition of photoreceptor inner segments, typically at a level slightly distal to where fins emerge (Fig. 6d). A representative gap junction joining a rod and a cone is shown in Figure 6d, and a higher magnification view of a photoreceptor gap junction illustrating the typical seven-layered structure is provided in Figure 6e.

In a survey of random vertical sections through the region of photoreceptor inner segments, we recorded the numbers and dimensions of every gap junction encountered. It was noted that rod-cone gap junctions ($n = 42$) were much less numerous than either cone-cone ($n = 158$), or rod-rod gap junctions ($n = 207$). The areas of the respective gap junctions were estimated by assuming that gap junctions were round, with a diameter equal to the longest profile seen in the electron microscope. Based on these assumptions the mean dimensions were estimated to be: rod-rod, $0.051 \mu\text{m}^2$; cone-cone, $0.029 \mu\text{m}^2$; rod-cone, $0.016 \mu\text{m}^2$. Photoreceptor gap junctions in the *Xenopus* retina were previously identified in freeze-fracture (Nagy and Witkovsky, 1981) from which a particle (presumed connexon) density of $4,270 \mu\text{m}^{-2}$ was measured. By using this value and the above dimensions, rod-rod gap junctions have 218, cone-cone gap junctions, 124, and rod-cone gap junctions, 68 connexons, on average. The relative numbers of gap junctions gathered from random sections, however, might be subject to

sampling bias. In particular, small junctions whose dimensions approach the section thickness might be missed, and this bias would lead to a relative underestimation of the number of rod-cone junctions. Random sections also do not provide reliable information about the distribution of gap junctions among neighboring receptors.

Therefore, as a more definitive probe of the pattern of gap junctions among rod and cone photoreceptors, a portion of the photoreceptor layer was sectioned serially through the inner segments, where the photoreceptor gap junctions were seen to occur. The sample area contained 54 receptors, consisting of 35 rods and 19 cones (Fig. 7). Figure 7 illustrates that virtually all rods are connected to other rods, and most cones are connected to other cones, whereas rod-cone contacts occur infrequently.

Model for rod-cone coupling

We have analyzed two patterns of connectivity, which can be seen in Figure 7 (top; outlined by boxes). These two regions are redrawn orthogonally below: The simpler one (Fig. 7, network A) contains a single point of rod-cone contact, and the second (Fig. 7, network B) contains two points. Our model is only semiquantitative, in that the values for the unitary gap-junctional conductance(s) and the open probability have to be estimated. These values vary widely from system to system (for review, see Bennett, 1997). It is also possible that rod-rod gap junctions have different elementary properties than cone-cone junctions. In the absence of specific values for the various photoreceptor gap junctions, we adopt the value of 50 pS for the unitary conductance, because it was obtained recently for a retinal horizontal cell gap junction (McMahon and Brown, 1994). The open probability is taken as 0.5. Errors in these values will affect the rate at which voltages fall as a function of distance from the point of initiation, but the model nevertheless provides an accurate picture of the properties of the network.

Based on the gap-junctional dimensions, a unitary conductance of 50 pS and an open probability of 0.5, we calculate the following values for coupling resistances: rod-cone, 0.588 GΩ; rod-rod, 0.183 GΩ; and cone-cone, 0.323 GΩ. The resistance to ground of the membrane of a single rod or a single cone was taken to be 1.0 GΩ (Torre and Owen, 1983). The cases that were considered were the limiting cases: 1) when cones were stimulated, but rods were not, and 2) when rods were stimulated, but cones were not. To the degree that the circuit is linear, i.e., no significant voltage-dependent changes in any of the conductances, the relative proportion of rod-generated and cone-generated components of the response of any cell can be estimated by simple addition. For example, linearity would be expected for small perturbations around the membrane potential.

The voltage of one cell relative to that of its neighbor can be found by using a difference equation. For example, in network A,

$$[(V_2 - V_4)/R_c]R' = V_4, \text{ giving } V_4 = \left[R'/R_c + R \right] V_2,$$

where R' is the effective input resistance of cell 2 seen from cell 4. Thus, the analysis also depends on calculating the input resistances of the various branches of the network. A chain of n cells has an input resistance that can be found by using a recurrence relationship:

$$(R_{in})_n = R_m [R_c + (R_{in})_{n-1}] / R_m + R_c (R_{in})_{n-1},$$

where R_m is the membrane resistance of a single cell (and the input resistance when $n = 1$), and R_c is the coupling resistance. The relative voltages experienced by each photoreceptor in the network for the limiting conditions of pure rod or pure cone input are provided in Table 2.

What these values indicate is that the apparent size of the cone input to a given rod depends not only on its proximity to a cone-rod gap junction and the number of such junctions but also on the number of rods to which the impaled rod is coupled (its “coupling domain”) and the number of cones in the coupling domain providing input to the rod. Given the multiple factors that influence rod-cone coupling, we expect to see a wide range of coupling strengths, which is consistent with our experimental data (cf. Fig. 1). It can be seen that, as a case in point, for the experimental condition in which only cones are light-driven, the cone contribution to the rod response varies by more than a factor of three (Table 2, lower left, V_1 – V_7).

DISCUSSION

By using electron microscopy, we found that rods are joined to neighboring rods through numerous gap junctions, giving rise to an extensive network of coupled rods. Cones are similarly connected into a cone network. Rod-cone coupling is mediated by gap junctions, which link about 10% of the rod population to cones. We noted that, even in nominally dark-adapted retinas that were superfused with normal Ringer's solution, rods showed varying degrees of cone input, as manifested by quickened response kinetics and a greater or lesser ability to follow sinusoidally modulated red light beyond 5 Hz (cf. Fig. 1).

The rods that we studied were 0.5–1.0 log units less sensitive than fully dark-adapted rods (Engbretson and Witkovsky, 1978). Attwell (1990) has shown that fully dark-adapted amphibian rods are 2 log units more sensitive than dark-adapted cones. Thus, the response range of the partially desensitized rods that we studied overlaps that of cones, particularly in relation to red stimuli. We provide a demonstration of movement of the tracer molecule, neurobiotin, into neighboring rods and cones from an injected rod, and we document the modulation of rod-cone coupling by dopamine. We determined that the dopamine-induced increase in rod-cone coupling is effected through a D2 dopamine receptor, whereas D1 dopamine ligands did not alter rod-cone coupling. The implication of a D2 dopamine receptor in rod-cone coupling is consistent with a receptor-binding study that localized D2 receptors to the photoreceptor layer in the *Xenopus* retina (Muresan and Besharse, 1993).

Presence and mechanism of rod-cone coupling in vertebrate retinas

Both anatomical and functional data support the presence of rod-cone coupling in a variety of vertebrate retinas, including those of mammals (Raviola and Gilula, 1973; Nelson, 1977; Schneeweis and Schnapf, 1995), reptiles (Schwartz, 1975; Copenhagen and Owen, 1976), and amphibians (Gold, 1979; Gold and Dowling, 1979; Nagy and Witkovsky, 1981; Wu and Yang, 1988; Yang and Wu, 1989). The results of current and dye-injection experiments strongly support electrotonic communication as the sole basis, or at least the primary basis, for direct rod-cone coupling, although there is anatomical evidence for chemical synapses between rods and cones in the salamander retina (Lasansky, 1973). In addition, a polysynaptic, sign-inverting pathway for rod-cone communication, using the horizontal cell as an intermediate, was demonstrated by Attwell et al. (1983).

Direct rod-cone coupling through gap junctions provides for rapid, sign-conserving signal transfer, whose magnitude is a function of the ratio of coupling resistance to the leak resistance to ground. In the retinas of the salamander (Wu and Yang, 1988) and *Xenopus* (present report), it appears that rods and cones are coupled into quasi-independent networks, which come into contact at relatively infrequent nodes. The data from examination of rod-cone coupling in the salamander (Wu and Yang, 1988) and the present study also agree that only a minority (10–

20%) of rods show strong cone input and that such rods are morphologically identical to the majority of rods.

We observed that, in normal Ringer's solution, the largest fraction of rods encountered was of subtype A and that subtypes B, C, and D were progressively smaller fractions of the total. This finding is consistent with our hypothesis that cones contact only type D rods, from which the cone signal is distributed into neighboring rods that lack direct coupling to cones. The D2 dopamine receptor increases coupling at rod-cone gap junctions, presumably by decreasing their resistance. Because the permeability of rod-rod gap junctions apparently was not modified by dopamine ligands, it is possible that the D2 receptor modulating the gap junction is located in the cone. A possible implication is that the cone network should be constricted by dopamine, but we made no direct measures of cone-cone coupling.

Although our study did not investigate the mechanism of coupling modulation, it may have resulted from a reduction of cytoplasmic (cAMP), which is a known mode of operation for D2/D4 dopamine receptors (Dearry et al., 1991; Cohen et al., 1992). Iuvone (1986) provided evidence that D2 receptors are implicated in the reduction of cAMP in *Xenopus* photoreceptors. The relation between electrotonic coupling and (cAMP) is inferred from numerous studies showing that an increase in (cAMP), mediated by D1 dopamine receptors, results in decreased electrotonic coupling between retinal horizontal cells (Piccolino et al., 1984; Lasater and Dowling, 1985).

The results of the neurobiotin injections indicate that rod-rod coupling is altered little by exposure to either quinpirole or spiperone, whereas rod-cone coupling is changed dramatically. In the *Xenopus* retina, exposure to light increases dopamine release (Boatright et al., 1989; Witkovsky et al., 1993), which presumably will result in increased rod-cone coupling in light-adapted retinas, as reported for the salamander retina by Yang and Wu (1989). Similarly, in the turtle retina, rod-cone coupling is greater when rods are slightly desensitized (Schwartz, 1975) compared with fully dark-adapted rods (Copenhagen and Owen, 1976).

Significance of rod-cone coupling for visual information processing

Rod-cone coupling is important in the mesopic state, when both rods and cones are light-responsive, and the electrotonic bridges between rods and cones permit one photoreceptor class to serve as a conduit for signals arising in the other class. In the cat retina, Nelson (1977) implicated rod-cone coupling in the transfer of rod information to horizontal cell bodies. In the rabbit retina, de Vries and Baylor (1995) found that, when the rod-to-“on-bipolar” cell pathway was blocked pharmacologically, rod signals still reached certain ganglion cells, presumably through rod-cone coupling.

In the *Xenopus* retina, rods become more cone-like in their response properties through two distinct, D2 dopamine receptor-mediated mechanisms. One mechanism reduces I_h , an intrinsic, voltage-dependent current in the rod that contributes to the rod transient (cf. Fig. 1) evoked by a bright flash (Akopian and Witkovsky, 1996). The other mechanism is the modulation of gap-junction permeability described in this study. It seems likely that rod signals also flow into the cone network across the same gap junctions, although we have no direct information on this point. We have emphasized cone input to rods, because the levels of dopamine in the *Xenopus* retina vary as a function of time of day and exposure to light (Witkovsky et al., 1993). Thus, in mesopic or photopic conditions, dopamine levels are high, but, when the retina is rod driven, at night or following prolonged dark-adaptation, dopamine levels are low, and the rod-cone gap junctions would have low conductance.

Because only a minority of rods receive direct cone input, during mesopic vision, the rod population is inhomogeneous in its response characteristics. In amphibian retinas, the second-

order retinal neurons, horizontal and bipolar cells, receive direct synaptic input from both rods and cones (Hare et al., 1986). Thus, during the transition from cone to rod vision, or the reverse, these cells will receive a diverse set of photoreceptor inputs, reflecting the presynaptic interactions among rods and cones. Whether or not inhomogeneous rod inputs are distributed randomly to second-order neurons and what the relative importance of rod-cone coupling might be in relation to direct synaptic contacts between photoreceptors and second-order neurons are topics for future study.

Acknowledgments

This work was supported by NIH grants EY 03570 (P.W.) and EY 03785 (W.G.O.); by the Hoffritz Foundation (P.W.); by OTKA grant F-16040 and a travel grant from Research to Prevent Blindness (R.G.); and by an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology, NYU Medical Center.

Grant sponsor: NIH; Grant numbers: EY 03570 and EY 03785; Grant sponsor: Hoffritz Foundation; Grant sponsor: OTKA; Grant number: F-16040; Grant sponsor: Research to Prevent Blindness.

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Fig. 1.

Rod responses to flashes and sinusoidally modulated light. Left: Intracellular recordings of rod responses to sinusoidal modulation of 567 nm log quanta ($\log Q$) incident $\text{cm}^{-2} \text{second}^{-1}$ 11.56 (green; grn) light or 660 nm $\log Q$ 13.63 (red) light. Rod types are labeled, and sine wave frequency is indicated below. For type A rods, note the similarity of responses elicited by red or green sinusoids. For type B rods, 8-Hz red response is greater than at 4 Hz. For type C rods, responses to green light are similar to type A rods, but red sinusoids > 2 Hz evoke relatively large responses. Type D rods show the greatest ability to follow red sinusoids. Right: Responses of rod types A and C to 567-nm (upper record of pair) and 667-nm flashes matched in intensity to elicit equal rod plateaux. Stimulus markers are indicated at bottom: type A rod, 300 msec; type C rod, 200 msec. Arrow indicates rod initial transient modified by cone input.

Fig. 2. Rod transients evoked by test flashes. With reference to the sample wave forms shown in Figure 1, right, the fraction of the total voltage (V_{max}) corresponding to the initial transient is plotted for rod types A–D. In every case, the stimulus was 100 msec, 660 nm, log Q 13.93. Vertical bars are $\pm$ SEM ($n = 86$).

Fig. 3.
a–f: Neurobiotin injections of single rods. Photomicrographs a–d show groups of neurobiotin-stained photoreceptors in retinal wholemount preparations. Up to eight stained receptors are visible. Arrows in c and d indicate cones. Stained photoreceptors are illustrated in e (cone; arrow) and f (rod) and are visible in 2-mm vertical sections. Scale bar = 20 μ m.

Fig. 4.

Effect of dopamine ligands on rod responses to sinusoids. Rods were classified into subtypes A–D on the basis of their responses to sinusoids, as illustrated in Figure 1. Rods were sampled at random during superfusion with normal Ringer's solution ($n = 92$) or with Ringer's solution to which $25 \mu\text{M}$ SKF 38393 ($n = 19$), $10 \mu\text{M}$ quinpirole ($n = 29$), or $5 \mu\text{M}$ spiperone ($n = 10$) was added. The graph axes, which are labeled only for normal Ringer's solution, are identical for each of the four graphs. Relative to normal Ringer's solution, quinpirole shifted the distribution toward types B, C, and D, whereas spiperone restricted it to type A. SKF 38393 was without statistically significant effect on the distribution of rod types.

Fig. 5.

Flash responses of a type D rod that was stimulated with 100-msec flashes of 567-nm light (open arrow) or 660-nm light (solid arrows). Stimulus markers are indicated at bottom. The profile of the rod after neurobiotin injection is indicated at right (**inset**). Top to bottom: Stimulus strength increased in 0.4 log unit steps. Note that, for the weakest pair, response to red light is faster than to green stimulus. Middle and bottom records show that, when stimuli are matched to elicit equal rod plateaux, the red flash elicits a larger transient. Scale bar = 20 μ m.

Fig. 6. Photoreceptor fins and gap junctions. **a:** Light microscopic view of a vertical section of *Xenopus* retina showing apparent contact points between photoreceptors (arrows). **b:** Tangential section through the photoreceptor inner segments. Both rod and cone profiles show radiating fins. **c:** Electron microscopic (EM) view of tangential section through photoreceptor inner segments. One pair of fins is indicated by arrows. Note that fins of neighboring photoreceptors do not make contacts. Small, round profiles are villous extensions of Muller glial cells. **d:** Low-magnification EM view of a gap junction (arrow) between a rod (R) and a cone (C). **e:** Higher magnification view of a rod-cone gap junction. Scale bars = 10 μm in b (also applies to a), 1 μm in c, 0.5 μm in d, 0.1 μm in e.

Fig. 7. Gap-junctional networks among *Xenopus* photoreceptors shown in schematic form indicating relative positions of rod and cone photoreceptors in a portion of reconstructed retina viewed in horizontal section. Cones are labeled with c, solid circles indicate are rods. Straight thick bars and thin bars indicate rod-to-rod and cone-to-cone gap junctions, respectively. Double arrowheads indicate rod-cone gap junctions. Irregular boxes delimit two groups of receptors that are redrawn orthogonally at the bottom and that were analyzed for coupling (see text).

TABLE 1

Neurobiotin Injections Into Rods

Condition	No. injections	No. stained rods	No. stained cones	No. stained cones/rod
Controls	21	111	11	0.10
Spiperone	10	55	0	0.00
Quinpirole	12	53	12	0.23

TABLE 2Effect of Photoreceptor Coupling on Signal Transmission in the Network¹

Only cones stimulated ²	Only rods stimulated ³
Network A	
$V_1 = V_3 = V$	$V_6 = V$
$V_2 = 0.91V$	$V_5 = 0.96V$
$V_4 = 0.38V$	$V_4 = 0.86V$
$V_5 = 0.28V$	$V_2 = 0.35V$
$V_6 = 0.24V$	$V_1 = V_3 = 0.26V$
Network B	
$V_{10} = V_{14} = V$	$V_1 = V$
$V_{11} = V_{13} = 0.97V$	$V_2 = 0.86V$
$V_{12} = 0.90V$	$V_3 = 0.85V$
$V_8 = 0.67V$	$V_4 = 0.88V$
$V_9 = 0.77V$	$V_5 = 0.73V$
$V_7 = 0.34V$	$V_6 = 0.74V$
$V_6 = 0.29V$	$V_7 = 0.69V$
$V_5 = 0.23V$	$V_8 = 0.30V$
$V_4 = 0.13V$	$V_9 = 0.28V$
$V_3 = 0.16V$	$V_{12} = 0.20V$
$V_2 = 0.12V$	$V_{11} = V_{13} = 0.13V$
$V_1 = 0.1V$	$V_{10} = V_{14} = 0.10V$

¹ Calculations of signal transfer between the photoreceptors diagrammed in Figure 7, lower portion (Fig. 7A is network A, Fig. 7B is network B).² Cone-generated voltages (V).³ Rod-generated voltages (V). Subscripts 1–11 refer to the particular rods and cones diagrammed in Figure 7.