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Mesopic State: Cellular Mechanisms Involved in Pre- and Post-Synaptic Mixing of Rod and Cone Signals

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Abstract

At least twice daily our retinas move between a light adapted, cone-dominated (*photopic*) state and a dark-adapted, color-blind and highly light-sensitive rod-dominated (*scotopic*) state. In between is a rather ill-defined transitional state called the *mesopic state* in which retinal circuits express both rod and cone signals. The mesopic state is characterized by its dynamic and fluid nature: the rod and cone signals flowing through retinal networks are continually changing. Consequently, in the mesopic state the retinal output to the brain contained in the firing patterns of the ganglion cells consists of information derived from both rod and cone signals. Morphology, physiology, and psychophysics all contributed to an understanding that the two systems are not independent but interact extensively via both pooling and mutual inhibition. This review lays down a rationale for such rod-cone interactions in the vertebrate retinas. It suggests that the important functional role of rod-cone interactions is that they shorten the duration of the mesopic state. As a result, the retina is maintained in either in the (rod-dominated) high sensitivity photon counting mode or in the second mode, which emphasizes temporal transients and spatial resolution (the cone-dominated photopic state). Experimental evidence for pre- and postsynaptic mixing of rod and cone signals in the retina of the clawed frog, *Xenopus*, is shown together with the preeminent neuromodulatory role of both light and dopamine in controlling interactions between rod and cone signals. Dopamine is shown to be both necessary and sufficient to mediate light adaptation in the amphibian retina.

Keywords

Xenopus; retina; gap junction; dopamine; photoreceptor; cAMP; adaptation; D2; D1; neuromodulation

What we need to know is by what arrangement can several types of receptors converge upon a ganglion cell and exhibit at the same time the characteristics and the very different properties seen in [the mesopic state].

—W.A.H. Rushton (1959)

INTRODUCTION

From the very earliest work by Schultze and von Kries it has been known that rods and cones divide their working ranges: daylight vision originates in cones whereas the night vision is sustained by rods. Such a functional division of retinal signaling into rod-dominated and cone-dominated portions was supported by the classical experiments of Aguilar and Stiles (1954) who showed a “break” between rod and cone increment threshold curves at backgrounds of

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around 6.10^4 quanta $(\text{deg}^2\text{s})^{-1}$. At this intensity, rods were therefore thought to desensitize and give way to the cone system (the “rod-cone break” or the “Purkinje shift”). According to the Aguilar and Stiles schema, the rod mechanism and the three-cone mechanisms function over largely non-overlapping ranges of average environmental brightnesses and, therefore, behave as independent and parallel units. The cone system was assumed to take over from rods after these were saturated by photons; conversely, rods were thought to take over from cones when the visual stimulus became too dim for adequate photon capture by the cones.

Early on, three lines of evidence put to question the independence of the rod and cone systems. The first originated from the studies concerned with the adaptive effects of steady activation of one system upon the sensitivity of the other (MacLeod, 1972; Sandberg et al., 1981). Psychophysical experiments showed that the rod-cone break occurred earlier with shortest wavelength stimuli, suggesting that cone-mediated signals may be under an inhibitory influence of the rod system. Now we know that when the rod system is light-adapted or bleached, the cone flicker threshold in the parafoveal retina can be lowered by as much as one log unit from its dark adapted value (Alexander et al., 1988). Indeed, an extensive mixing of rod and cone signals has been recently demonstrated in both rabbit (Xin and Bloomfield, 1999) and primate (Stone et al., 1997; Verweij et al., 1999) retinas. Secondly, Steinberg reported in 1969 that the light response from mesopic cat horizontal cell bodies consists of both rod and cone signals (Steinberg, 1969). Since the cat HC body does not contact rods directly, it was proposed that the rod signal contacts the HC was via the cone synapse through cones electrically coupled to rods. This hypothesis was substantiated a few years later when gap junctions were identified between rods and cones (Nelson 1977, Raviola and Gilula, 1973). Finally, it was the realization that in duplex retinas rod and cone signals converge onto the same final common pathway—the ganglion cell—before exiting the eye (Enroth-Cugell et al., 1977; Rodieck and Rushton 1976) that paved the way for a more comprehensive view of the mesopic state. The inevitable conclusion of the early work was that during the mesopic state, signals emanating from both rod and cone systems share the same retinal circuits. We now know that interactions between rod and cone signals occur in all species possessing duplex retinas (including amphibians, reptiles and mammals) at virtually every level of retinal signal processing (Alexander et al., 1988; Arden and Hogg, 1985; Dong et al., 1988; Frumkes and Eysteinnsson, 1988; Goldberg et al., 1983; Hassin and Witkovsky, 1983; Schwartz 1975; Stockman et al., 1995; Witkovsky et al., 1988). Moreover, they occur within a significant range of behaviourally relevant environmental light intensities: many visual stimuli effective for rods can also activate cones (e.g., Wu, 1994), and vice versa. At the moment, many of the mechanisms by which the signals from different photoreceptors interact are still unknown and the biological significance of the interactions is not completely understood. By observing and dissecting these mechanisms in the amphibian preparation, we may have a usable tool to understand cellular mechanisms involved in such interactions. This review will illustrate some general mechanisms of rod-cone interactions that exist in the outer plexiform layer (OPL) at the first synapse in the *Xenopus* retina. It will be shown that rod and cone signals mix both via electrical junctions between photoreceptors themselves as well as via 2nd messenger cascades in the postsynaptic cells. Many of these events are under neuromodulatory control with the catecholamine dopamine playing a central role.

XENOPUS EYECUP PREPARATION AS A TOOL TO STUDY ROD-CONE INTERACTIONS

Cellular mechanisms responsible for rod-cone interactions in the OPL are best studied by recording responses from retinal cells in a functioning network. This, however, also presents a potential limitation, since such work requires a relatively intact preparation, with interactions between (1) the photoreceptors themselves; (2) photoreceptors and retinal pigment epithelium

(RPE) cells; (3) photoreceptors and second-order horizontal cells (HCs) and bipolar cells (BCs) as well as (4) photoreceptors and the cells in the inner plexiform layer such as the catecholaminergic and peptidergic amacrine and the glycinergic interplexiform cells. Retinal slices, for example, are usually prepared with the RPE sheet torn off the photoreceptors. Slice recordings are consequently conducted under conditions that prevent recovery of the bleached photopigment and thus lack the reversibility of the respective rod/cone system gain mechanisms. Ideally, therefore, one should work on an eyecup preparation or in the intact eye. Another consideration pertains to the species studied. Recording from primate retinas is fraught with experimental and logistic difficulties. The retinas are difficult to obtain and difficult to maintain. On the other hand, many of the fundamental problems in retinal signaling can be profitably tackled by using preparations from cold-blooded animals, which are cheap, easy to maintain, and often consist of large cells (a notable example are the urodele retinas such as those of the tiger salamander and the mudpuppy). Elements of rod-cone interactions, such as the tonic suppression of the cone system by the dark adapted rod system, are observed both in amphibian and human retinas (Alexander et al., 1988; Dong et al., 1988; Frumkes and Eysteinnsson 1988; Hassin and Witkovsky, 1983; Hood, 1972; Sandberg et al., 1981). In this review, I will show that the *Xenopus* preparation possesses several advantages that can yield important insights about rod-cone interactions and retinal function in general. The experiments presented below were all performed in the laboratory of Paul Witkovsky who has pioneered the study of rod-cone interactions and dopaminergic modulation of synaptic transmission in the outer retina (e.g., Witkovsky et al., 1988, 1997; Witkovsky and Shi, 1990; see also Witkovsky, pages 338–346, this issue).

- The morphology of the *Xenopus* retina as well as basic physiological mechanisms have been extremely well characterized (Schütte and Witkovsky, 1991; Wilhelm and Gabriel, 1999).
- The OPL in *Xenopus* is relatively simple compared to other vertebrate retinas. It contains a dominant green-sensitive rod and a dominant (90%) red-sensitive cone photoreceptor (see reviews by Witkovsky, pages 338–346, Röhlich and Szél, pages 327–337, and Gábel, pages 394–402, this issue). Yet the basic structural and functional organization of the amphibian retina is fundamentally similar to the mammalian/primate and many essential features of the *Xenopus* retina resemble those of the phylogenetically more “advanced” vertebrates (e.g., Hack et al., 1999). The spatial resolution power in the *Xenopus* retina may be similar to that found in the central retina of mammals (Wilhelm and Gabriel, 1999). Consequently, many principles of retinal function in the *Xenopus* retina, particularly those operating at the level of cellular ensembles, can be applied profitably to other phyla.
- *Xenopus* is a cold-blooded species, permitting use of eyecups that remain viable for long periods of time at room temperature (in a typical experiment, the responsiveness of retinal neurons remains completely normal for >15 hours) while using relatively simple experimental setups. Recordings are stable and single rods and horizontal cells can be held for extraordinary lengths of time. Furthermore, synaptic transmission can be studied in long-lasting experiments with two electrodes simultaneously recording responses from cell pairs (Krizaj and Witkovsky, 1993). The *Xenopus* preparation is amenable to intracellular recording (Stone and Witkovsky, 1984), patch clamping (Akopian et al., 1997), microspectrophotometry (Witkovsky et al., 1981), and glutamate release studies (Schmitz and Witkovsky, 1997).
- *Xenopus* are available year round and can be purchased and maintained easily and inexpensively in aquaria for indefinite periods of time.

- The formidable tools of the transgenic technology are increasingly applied to *Xenopus* (eg., Fini et al., 1997; Smith, 1996). This will lead to exciting interdisciplinary projects in physiology and molecular biology.
- The *Xenopus* retina has proven to be a good preparation for the study of the effects of second messengers and neuromodulators in living intact tissue (see below).
- A major drawback for intracellular recording from the *Xenopus* eyecup is that, unlike in tiger salamander, it is extremely difficult to record intracellularly from cone photoreceptors. In the many years that I have recorded from this preparation, I may have impaled a cone two or three times and could never hold them more than a few tens of seconds. This problem may be obviated in part by complementary work on the closely related tiger salamander retina.

OUTLINE OF ROD-CONE INTERACTIONS

Rod and cone signals mix at virtually every level of retinal organization:

1. presynaptically via electrical contacts that connect rod and cone inner segments and/or synaptic terminals
2. postsynaptically via mutual shunting
3. postsynaptically through intracellular modulation by 2nd messengers
4. transynaptically via release of neuromodulatory chemicals such as dopamine, melatonin, or somatostatin

ROD AND CONE SIGNALS MIX PRESYNAPTICALLY

Intracellular recording from vertebrate photoreceptors under mesopic conditions shows that rods express a significant amount of cone input that shapes their spectral sensitivity as well as responses in the time domain (Attwell et al., 1984; Yang and Wu, 1989). Cone inputs in green-sensitive rods are best evoked with long wave stimuli that excite both rods and red-sensitive cones (e.g., Schwartz, 1975). The reverse situation also holds: rod signals can be recorded from mesopic cones (Nelson, 1977). The primary conduit for the pre-synaptic mixing of rod and cone signals are gap junctions that exist between photoreceptors in virtually all vertebrate species (reviewed in Gold, 1981; Cook and Becker, 1995; Krizaj et al., 1999). Although the identity of the connexins forming these electrical synapses is as yet unknown, the junctions themselves have been well characterized morphologically. In *Xenopus*, the gap junctions between rods and cones are formed directly between the respective inner segments (Fig. 1). Their area is $\sim 0.016 \mu\text{m}^2$, consisting on average of ~ 70 connexons, which is about 3 times less than junctions between rods alone (Krizaj et al., 1998).

Not all rods are connected equally well to cones. A wide range of spectral sensitivities and fusion frequencies is seen in mesopic rods recorded under identical conditions, suggesting that some rods receive more cone input than others (Fig. 2). When these rods are labeled with neurobiotin and examined under the electron microscope, their morphology turns out to be virtually identical to the “normal” green-sensitive rods (Krizaj et al., 1998); hence, it is possible that they are distinguished from the majority rods simply by a higher connexon density. These “gatepost” rods redistribute the cone signal into the rod network (Krizaj et al., 1998; Wu and Yang, 1988). While the function of the gatepost rods (which may comprise about 10% of rod population) is not known, it is possible that they help increase temporal resolution of the retinal network by feeding the rod signal into the cone network.

The flicker data and comparison of small amplitude responses to green and red stimuli suggest that even in the dark adapted state, the cone input to rods may be significant and that, therefore,

the principle of univariance does not hold even under these conditions (Krizaj and Witkovsky, 1993; Krizaj et al., 1998). The rod-cone coupling is modulated by background light; when the intensity of the background light is increased, coupling becomes stronger (Yang and Wu, 1989). Recent work in *Xenopus* has revealed that this effect is likely caused by the neuromodulator dopamine (Krizaj et al., 1998). Dopamine binds to D2/D4 receptors located in inner, and possibly outer, segments of rods and cones of many species, including *Xenopus* (Derouiche and Asan, 1999; Muresan and Besharse, 1993). Subsequently, dopamine initiates a signaling cascade that ultimately results in the opening of junctions between rods and cones. In addition to modulating gap junctions, dopamine controls a variety of other signaling pathways in photoreceptors, including the non-selective I_h cation channel, high voltage-activated Ca^{2+} channels, and various cytosolic enzymes (see also Akopian, pages 403–410, this volume). This action of dopamine is likely via the heterotrimeric G proteins such as G_i and G_o , which in turn modulate photoreceptor $[cAMP]_i$ (Cohen et al., 1992) and/or $[Ca^{2+}]_i$ (Akopian and Witkovsky, 1996; Sibley, 1999; Stella and Thoreson, 1998; Krizaj and Copenhagen, unpublished observations). Another, if less explored, D2-dopaminergic action may be modulation of release of the synaptic transmitter via regulation of $[Ca^{2+}]_i$, $[cAMP]_i$, or $[IP3]_i$.

Although the emphasis in this paragraph is on the electrical aspect of the interaction between rods and cones, it is important to emphasize that there may be other presynaptic venues of communication between these two photoreceptor classes. For example, in addition to redistributing the electrical signal, gap junctions may also gate the spread of 2nd messengers between the inner segments and synaptic terminals of rods and cones. The diffusional lengths for cAMP and IP3 are large (220 and 17 μm , respectively; Allbritton et al., 1992) making interaction via diffusible factors quite likely (see, for example, Copenhagen and Green, 1986, for some early suggestive evidence). Rods and cones also communicate via glutamate that they release at synaptic terminals. Rod terminals are often observed to invade cone pedicles (Mariani and Lasansky, 1984; Yang and Wu, 1997), suggesting a chemical synapse exists between the two classes of photoreceptors. Is it possible that the mGluR1 and mGluR8 metabotropic receptors recently described in synaptic terminals of rods in several species, including rat (Koulen et al., 1999) and cat (Cai and Pourcho, 1999) are the postsynaptic elements at such interphotoreceptor synapses?

In conclusion, photoreceptors are the first site in the retina that shows a dynamic regulation by neuromodulation. In the mesopic state, when all rods collect and temporally sum more than one quantum per integration time, that is, when illumination may be too dim for cones and too strong for rods, the rod-cone junctions open, allowing the cone signal with its high temporal resolution the use of the rod network that has a higher gain and channeling the robust rod signal into the cone pathway. On the other hand, in the dark adapted state when it is important for 2nd-order cells to detect dim signals, weak coupling between rods and cones prevents the shunting of the rod signal into the cones and thus gives the synapse greater sensitivity to light. Therefore, the gain between rods and postsynaptic cells is high under these conditions (Witkovsky et al., 1997). Neither light nor dopamine modulates junctions between rods (Krizaj et al., 1998; Yang and Wu, 1989), indicating that the modulation of electrical synapses between rods and cones is specifically related to light adaptation.

ROD AND CONE SIGNALS MIX POSTSYNAPTICALLY

Amphibian (i.e., *Xenopus*, *Rana*, *Ambystoma*, *Necturus*) luminosity horizontal cells (L-HCs) and bipolar cells (BCs) receive converging synaptic inputs from both rods and cones. Rod and cone systems activated during the mesopic state can be distinguished by their respective spectral sensitivities and kinetics of light responses measured from the optic nerve, the ERG, or individual cells. The contribution from the two respective photoreceptor classes is, for

example, clearly seen in the HC light response. Figure 3 shows a simultaneous intracellular recording from a mesopic rod and a HC pair.

While the rod component dominates the HC responses to dim 527-nm flashes (as seen by the similarity of their respective waveforms, Fig. 3Aa–c), a bright flash (Fig. 3Ad) evokes a strong cone component in the HC. When the flash responses in c and d are scaled and matched for the “tail components” contributed by the rod signal, two elements are noteworthy:

1. in the time course of the HC repolarization in Figure 3Ad is markedly different from the time course of the rod.
2. the rod component in the light responses to the brighter flash (Fig. 3Ad) is smaller from the response to dimmer flashes (Fig. 3Ac).

The suppression of the rod signal by the cone signal is shown in more detail in Figure 3B. In response to a dimmer flash, the light responses of the rod and the response of the HC are very similar (Fig. 3B, c₁). In fact, were it not for the cone-mediated “nose” (see arrow), the HC light response would be exactly superimposed onto the rod light response. This suggests that a large range of the presynaptic rod voltage change is transmitted quasi-linearly to the HC, certainly more than the few mV proposed by Attwell et al. (1987). The rod-HC response match changes when the preparation is exposed to a higher intensity flash. Now, as shown in the Figure 3B panel d₁, *the cone “nose” is increased* whereas, simultaneously, *the rod signal in the HC is dramatically depressed without the response of the rod itself being appreciably altered*. Not only is the magnitude of the rod component in the HC decreased but note that the kinetics of the HC light response also changes with an increase in the cone input. This suggests that the reduction and eventual complete disappearance (with bright flashes) of the rod signal in HCs cannot be explained by a simple saturation of the rod system or a shunt of the rod signal in the postsynaptic cell. Rather, it is likely that cone-activated intracellular mechanisms within the HC actively suppress the rod signal in the period during which the release of glutamate from rods is still suppressed. In the mesopic state, therefore, *a postsynaptic intracellular inhibitory signal activated by the cone pathway acts to reduce the gain of the rod-HC synapse*. The section below provides evidence that the cAMP pathway, activated by the dopamine D1 receptor, participates in the creation of such inhibition.

DOPAMINE CONTROLS TRANSITION FROM ROD-DOMINATED TO CONE-DOMINATED VISION

Retinal dopamine is synthesized by the dopaminergic amacrine cell, a relatively sparse, uniquely identifiable type of tyrosine hydroxylase-positive cell whose processes ramify in the sublayers 1, 3, and 5 of the IPL (Witkovsky and Schütte, 1991). In *Xenopus*, some processes emanating from this cell do reach the OPL but do not branch there, and it is likely that most of the released dopamine reaches its targets by diffusion from the IPL (Bjelke et al., 1996; Witkovsky et al., 1993). In light-adapted eyes, the retinal concentration of dopamine reaches $> 0.5 \mu\text{M}$, which is enough to dramatically alter the balance between rod and cone signals (Boatright et al., 1989; Krizaj and Witkovsky 1993; Witkovsky et al., 1993). We now know that *dopamine is both necessary and sufficient to light adapt the scotopic retina* (Krizaj and Witkovsky, 1993) and conversely, by adding D1 and D2 dopamine receptor blockers to a light-adapted retina, it is possible to show that retinal cells within minutes adopt a phenotype typical of the dark adapted state (Krizaj and Witkovsky, 1993; Witkovsky et al., 1988, 1989). The action of dopamine is both presynaptic via the D2/D4 dopamine receptor and postsynaptic via the D1 dopamine receptor (Krizaj and Witkovsky, 1993; Krizaj et al., 1994, 1998). What is the physiological stimulus for dopamine release? One important insight provided by the work of Witkovsky and Shi (1990) is that the weak, rod-effective light at dawn is already enough to turn on dopamine release. Now we know that light stimulates dopamine synthesis (by up-

regulating both the TH enzyme and its gene) as well as its release. The finding by Witkovsky and Shi thus allows us to visualize an elegant circuit: at dawn rods activate ON bipolar cells that, in turn, trigger release of dopamine from the TH-positive amacrine cells. Dopamine subsequently diffuses to the OPL, where it activates the cone circuits.

A fundamental rule that holds for all duplex retinas examined so far is that *only cells that are in contact with cones respond to dopamine*. This rule has been confirmed both by immunohistochemistry and through biochemical and physiological experiments. This is true for amphibian HCs, which receive inputs from cones and rods (Witkovsky et al., 1988) as well as for teleost HCs, which are connected to cones only (see Fröhlich et al., 1995). Thus, teleost cone-HCs possess D1 dopamine receptors and respond to dopamine (Dowling et al., 1983) whereas rod-HCs do not respond to DA and neither do they contain dopamine receptor of any class (Qian and Ripps, 1992). Similar results were observed in the mammalian retina (Veruki and Wässle, 1996).

Activation of D1 receptors causes the HC to display all the signs characteristic of photopic state: spectral sensitivity that corresponds to red cone pigment (Witkovsky et al., 1988) and flicker response that mimicks flicker responsiveness of cones, but not rods. This is shown in Figure 4.

Figure 4 illustrates a HC stimulated with either 200 ms steps of 650 nm light or ramps of red and green flicker. The cell light response under control conditions is shown in Figure 4A. The flash response is typical of rod-dominated mesopic responses: it consists of a small cone-mediated “nose” followed by a prolonged rod-mediated “tail.” The responses to both red and green flicker saturate around 6 Hz, again indicating that the rod signal predominates. A 15-minute exposure to the D1 agonist SKF 38393 has several major effects on the HC light response: (1) it eliminates the rod component of the flash response, (2) it potentiates the response to red flicker at frequencies >15 Hz, and (3) it completely inhibits the HC response to green flicker. Note that under these same conditions SKF 38393 has no effect on rod responses to either steps or flicker of light. The suppression of the rod signal in the HC could, therefore, result from D1 modulation of the rod synapse (e.g., modulation of I_{Ca} in the rod synaptic terminal) or it could be a postsynaptic effect whose explanation must be found in signaling mechanisms within the HC itself. The former hypothesis is rendered less likely, however, by immunohistochemical studies that show that D1 receptors in the outer plexiform layer are localized to the horizontal and bipolar cells (Veruki and Wässle, 1996). This suggests a postsynaptic function for the D1 dopamine receptor.

Xenopus HCs contact both rods and cones via ionotropic KA/AMPA receptors (Krizaj et al., 1994). The same is true for the *Xenopus* OFF BCs (Krizaj, 1995). Since both inputs are of the same (excitatory) sign, how can the apparently contradictory postsynaptic action of dopamine on the cone signal (potentiated) and the rod signal (suppressed) be explained? One possibility is that the dendritic tips contacting cone pedicles and rod spherules contain different subunits of GluRs. Another, not mutually exclusive explanation is that only the HC (and BC) dendrites contacting cone pedicles, but not rod spherules, possess D1 dopamine receptors. As a consequence, dopaminergic potentiation of the cone input could shunt the comparatively smaller non-potentiated rod signal (Witkovsky et al., 1989). The great variability between the relative rod/cone input between different L-HCs recorded under the same adaptational condition (Krizaj, unpublished observation) may, therefore, be due to different densities of D1 receptors or D1R-associated proteins.

Although the shunt hypothesis may explain a dopamine-mediated change in the amplitude, it cannot account for the large difference in the kinetics between the mesopic and scotopic rod signals measured in the HCs. One alternative explanation that takes into account the kinetics

changes rests on the neuromodulatory nature of dopaminergic action. The D1 mechanism is known to be coupled to adenylate cyclase and/or the phospholipase C and thus may exert its action by either an increase in [cAMP] and/or IP3/DAG (Pfeiffer-Linn and Lasater, 1996, reviewed in Sibley, 1999; Witkovsky and Deary, 1991). I suggest that the kinetic changes in the HC light responses are caused by the D1-dopamine receptor mediated activation of intracellular signaling mechanisms (see below). In classical experiments during the early 1980s, Dowling's group showed that dopamine elevates [cAMP]_i in the teleost HC, which in turn activates the protein kinase A (PKA; Dowling et al., 1983). This work suggested that the biochemical signature in HCs exposed to dopamine is dramatically changed compared to naive cells. Is it possible that the effect of the D1 agonist on the rod-cone balance in the HC occurs via cAMP cascade? Following exposure to dopamine, levels of cAMP in horizontal cells are increased (Young and Dowling, 1989), which may influence several adaptation-related mechanisms, including receptor desensitization and regulation of glutamate receptor-gated channels (Knapp and Dowling, 1987). We addressed this question by injecting cAMP in one of two simultaneously recorded dark adapted HCs. Indeed, as shown in Figure 5, following the injection of cAMP both the kinetics and sensitivity of the cell started to resemble those of light adapted, cone-dominated cells.

Figure 5 shows two simultaneously recorded dark adapted HCs. Following iontophoretic injection of cAMP, the waveform of the injected cells became much faster and cone-like. An even more suggestive result is shown in Figure 6. When two HCs are recorded simultaneously, the injection of cAMP into one cell radically changes its kinetics. The injected cell is less sensitive to dim flicker (Fig. 6A), whereas its responsiveness to bright flicker is enormously potentiated (Fig. 6B), suggesting that it is dominated by cone inputs rather than rod inputs. Note also that exposure to the bright light saturates the control HC (as evidenced by the half-wave rectification typical for saturated HC flicker responses) whereas the cAMP-injected cell oscillates around a mean membrane potential. Taken in toto, the effect of injecting cAMP is identical to that obtained by the application of the D1 receptor agonist (see Fig. 4) or by adapting the retina with strong background light.

This experiment strongly suggests that *DA acts as a postsynaptic switch modulating the balance between the rod and cone pathways* and that intracellular 2nd messengers can play an important role in regulating the relative weight of rod and cone information in retinal cells. Indeed, an elevation of [cAMP] in the HC is sufficient to light adapt its response phenotype.

The cAMP signaling machinery may be localized to the cone-connected HC dendrites via A-kinase anchoring proteins (AKAPs) that target the PKA to a defined intracellular location in a number of tissues (reviewed by Fraser and Scott, 1999). Targets for AKAPs include AMPA receptors and L-type calcium channels. Several questions may be posed with respect to these interesting proteins. For example, are AKAPs localized to HC dendritic branches contacting cones? Are these proteins forming macromolecular complexes with the AMPA receptors, dopamine D1 receptors, and the P/L-type calcium channels that are instrumental in shaping the HC and BC light response (Akopian et al., 1997; Krizaj et al., 1994; Maguire and Werblin, 1994; Pfeiffer-Linn and Lasater, 1996)? Another possible target of AKAPs may be the intracellular calcium stores in HCs (Micci and Christensen, 1998). Endoplasmic reticulum containing these stores is often located very close to the plasma membrane and a functional connection between the stores and the plasma membrane may be important for controlling the excitability and light responsiveness of the cell. Unfortunately, at the moment we know nothing about these proteins in the retina.

Both dopamine and D1 dopamine agonists also depolarize the HCs, consistent with increasing the amplitude of the glutamate-gated current (the reversal of which is at 0 mV; Krizaj et al., 1994). This effect of dopamine is probably achieved through the dopamine-mediated

facilitation of the glutamate-induced current flow through *AMPA receptor-gated channels*. The phenomenon was first described in teleost HCs by Knapp and Dowling (1987) and later observed also in *Xenopus* (Krizaj et al., 1994) as well as in tiger salamander (Maguire and Werblin, 1994) cells.

Figure 7 shows an example of the Knapp-Dowling effect in the *Xenopus* HC. When application of kainate (an agonist of the AMPA receptor) is preceded by a puff of 0.5 μ M dopamine, a doubling of the kainate-evoked current is observed. This potentiation lasts for several minutes, which is consistent with effect of DA at other central synapses. Note that the concentration of dopamine used in Figure 7 (0.5 μ M) mimicks [dopamine] measured in light adapted retinas (Witkovsky et al., 1993) and found experimentally to light-adapt the HC light responses (Krizaj and Witkovsky, 1993). In conclusion, dopamine has two complementary effects on HCs: it (1) *decreases* the amplitude of rod signals and it (2) *increases* the amplitude of the cone signals. Part of dopamine's action includes a potentiation of the excitatory currents flowing through the postsynaptic AMPA receptors located at cone:HC synapses. Additional effects of dopamine may include modulation of gap junctions, calcium channels, intracellular calcium stores, and GABA receptors (Pfeiffer-Linn and Lasater, 1996; Witkovsky and Dearry, 1991). Dopamine controls the balance of rod and cone signals in HCs at least partly through [cAMP], which apparently acts as an intracellular "switch" between scotopic and photopic cytosolic milieus.

WHY IS THERE A NEED FOR INHIBITORY ROD-CONE INTERACTIONS?

One problem inherent in the structure of the retina is that retinal circuits did not evolve separate rod-dedicated and cone-dedicated pathways. This is particularly evident in lower vertebrates, where bipolar and horizontal cells receive direct rod and cone inputs, but is a fact of life for mammalian retinas too, especially in the light of recent evidence (see, for example, Hack et al., 1999; Sharpe et al., 1999; Stone et al., 1997; Verweij et al., 1999; Xin and Bloomfield, 1999). Thus, when the retina is in a transition from a cone-dominated to a rod-dominated state of adaptation, most retinal channels are simultaneously filled with both rod and cone signals. This presents a timing problem: if both rods and cones respond to the same visual stimulus, then the retinal output would represent the same object twice: first with a fast, cone-mediated signal followed by a slower, rod mediated signal (this integration might be performed by the retinal ganglion cells, which simultaneously process "fast" signals through AMPA receptors and "slow" signals through the NMDA receptors; Diamond and Copenhagen, 1993). It is probably disadvantageous for an animal to receive double-latency information concerning the same even/object. This might, for example, result in an ambiguity of target's location and movement across the retina. Alternatively, an interference between the signals might lead to an artificial enhancement/destruction of the signal at certain temporal frequencies (see, for example, the destructive interference between rod and cone flicker recorded from *Xenopus* rods in Krizaj et al., 1998; another example is seen in cat X-cells, the center and surround response of which undergo a sudden phase lag due to destructive interference at ~0.5 cat scotopic td). The mutual antagonism between the rod and cone signals decreases the magnitude of this problem as whichever system is more strongly activated will exert a powerful suppressive influence upon the other. This would preserve the integrity of timing. Such interaction between rod and cone signals was indeed demonstrated in cat, where the rod and cone systems may inhibit each other by at least 30% (Levine et al., 1987) and in mesopic *Xenopus* HCs, where a suppression of the cone-HC synapse disinhibits the rod-HC synaptic signal (Krizaj et al., 1994). With respect to adaptation, such a *tug of war between the two types of signals serves to shorten the mesopic range*: even though the weaker system is neither subthreshold nor saturated it is not expressed because it is inhibited by the stronger signal. The mechanism of the inhibition involves, at least in part, the neuromodulator dopamine, which potentiates the cone signal and suppresses rod signals in a variety of retinal cells, including horizontal cells, bipolar cells, and ganglion cells (Krizaj, 1995). Hence, although they are still

active in responding to light, rods are prevented from sending a signal down the optic nerve. Thus the end result of the dopaminergic suppression of the rod signal is that the retina is pushed still further into the photopic state.

An experiment that supports this line of reasoning is shown in Figure 8. Here flicker responses were recorded intracellularly from a mesopic L-HC under two highly artificial conditions (Aguilar and Stiles, 1954): a green background (to suppress rod signals) and red background (designed to suppress cone signals). When the HC was exposed to a rod-effective green background, the cell responded quasi-sinusoidally up to 15 Hz in a manner typical for cone signals. When, however, the cone inputs were suppressed with a cone effective (650 nm) background, a frequency doubling can be observed in the HC flicker response.

The waveform of the HC shows a short-latency peak corresponding with the cone-driven peak in response to the green background and a slower-latency peak that is suppressed in the green background and that disappears at 4–5 Hz, which is the fusion frequency for mesopic rods (see Fig. 2).

A similar phenomenon with an opposite sign, that of rods suppressing the cone system (e.g., Goldberg et al., 1983), acts to shut the cones off the dark adapted state. The mechanism that underlies this rod-mediated suppression of cone signals is still completely unknown. Perhaps not surprisingly, this effect is diminished by dopamine (Frumkes and Eysteinnsson, 1988).

What, if any, role do rod-cone interactions have in determining the behaviour of the organism is presently not known. However, the fact that the sensitivities of individual rod and cone receptors measured physiologically are far closer to one another than are the rod-mediated and cone-mediated behavioural thresholds (Hood, 1998) is strongly suggestive of the possibility that postreceptoral interactions between the two signaling pathways contribute to behaviourally relevant perception. In addition to the classical notion of synaptic adaptation at the photoreceptor synapse, the role of which is to prevent saturation and thus maintain coding in the region of the highest sensitivity (Laughlin, 1989), we may now add suppression of the weaker pathway resulting in a shortening of the mesopic state as another mechanism that contributes to maintaining the retina in an optimal functional state.

CONCLUSION

The action of dopamine and the action of light in the amphibian retina (Krizaj and Witkovsky, 1993; Witkovsky et al., 1988) are nearly identical, suggesting that dopamine acts as a switch to turn on the photopic state and shorten the duration of the mesopic state. Under dark-adapted conditions, retinal dopamine concentration is low (~150 nM) and the retina is dominated by rod signals. An increase in ambient light has several consequences: (1) it light adapts rods proportionately more than cones, resulting in a larger proportion of glutamate released from cones; (2) it stimulates dopamine release from TH-positive amacrine cells. Subsequently, dopamine acts at several sites in the retina to potentiate the cone signal and suppress the rod signal. One action of dopamine is to facilitate electrical coupling between rods and cones. Another is the potentiation of the excitatory transmission at the cone-HC synapse through both an enhancement of the current flow through the cone transmitter-gated channels and a suppression of the rod signal via an unspecified intracellular 2nd messenger cascade. The suppression is initiated by the D1 dopamine receptor and may, at least in part, involve a rise in $[cAMP]_i$. It is interesting to note that dopamine plays an opposite yet complementary role in intracellular signaling of photoreceptors and HCs: the binding of dopamine to the D1 receptor in HCs *increases* $[cAMP]_{HC}$ resulting in a *closure of the gap junctions between neighbouring HCs*. On the other hand, activation of the D2 receptor in the photoreceptors by dopamine *decreases the* $[cAMP]_{photoreceptor}$ *and opens the gap junctions between rods and*

cones. Thus dopamine may increase the temporal resolution by opening gap junctions between rods and cones and the spatial resolution by closing the gap junctions between coupled HCs and amacrine cells. Similar parallel modulation of junctional coupling by D1- and D2-like mechanisms may also occur in other areas of the CNS, such as the nucleus accumbens (O'Donnel and Grace, 1995).

The overall conclusion is that synaptic transmission at the photoreceptor output synapse is fixed neither presynaptically nor postsynaptically. Rather, a highly complex and dynamic pattern of extracellular modulators, receptor proteins, and intracellular signaling cascades results in an adaptable and ever shifting functional state that is optimized for the behavioural action.

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Fig. 1.

A: Electron microscopic view of a gap junction between inner segments of a rod (R) and a cone (C). Scale bar is 0.5 μm . **B:** Higher magnification of a rod-cone gap junction. Scale bar = 0.1 μm .

Fig. 2.

Intracellular recording from rod photoreceptors. **Aa:** "Ordinary" rod responses to green (567 nm) and red (667 nm) steps of light matched for equal absorbance by the rods. Stimulus marker is 300 msec. **Ab:** Responses to sinusoidally modulated green (567 nm; 11.56 log quanta $\text{cm}^{-2} \text{s}^{-1}$) and red (660 nm; 13.63 log quanta $\text{cm}^{-2} \text{s}^{-1}$). Cells were exposed to a flicker ramp with frequencies from 1 to 8 Hz. Note that the fusion frequency for this cell is below 4 Hz for both red and green stimuli. **Ba:** "Gatepost" rod responses to green and red steps of light. Note that the red, but not the green, flash evokes a transient response in the rod strongly resembling the cone light response (arrow). Stimulus marker is 200 msec. **Bb:** Flicker responses of the gatepost rod peter out before 4 Hz whereas responses to red flicker are maintained even at 8 Hz.

Fig. 3.
Aa–d: Simultaneous recording from a rod and an L-HC in the mesopic state (9.88; 10.72; 11.55; 12.38 logQ). Stimulus markers are 200 msec. Note that the strongest flash induces a suppression of the rod tail in the HC but not in the rod itself (arrow). **Bc₁–d₁:** Scaled and superimposed responses from Aa–d. Whereas the responses of the rod and HC in c are remarkably similar (note in particular the kinetics of the onset and the offset of the cells' light responses), both their amplitude and kinetics are dramatically changed with the stronger flash in d/d₁.

Fig. 4.

The effect of the dopamine D1 agonist SKF 38393 on the HC. **Left:** Responses of an HC in the rod-dominated mesopic state to 200 msec 650 nm flash (12.0 logQ) before (A) and after (B) exposure to SKF38393. The waveform of the cell under control conditions shows a slow afterhyperpolarizing tail that is abolished by SKF. **Right:** HC flicker responses to green (567 nm) and red (660 nm) flicker 1–15 Hz ramps. Under control conditions, the HC in A responds to both red and green flicker to ~ 5Hz. Following exposure to 40 μ M SKF38393, the response to green flicker in the same cell is completely suppressed, whereas the red flicker follows well up to 15 Hz. Note that in A the cell hyperpolarizes to its V_{max} when hit by the flicker, whereas after SKF treatment it repolarizes to about the middle of its operating range.

Fig. 5. Simultaneously recorded HC pair. **A:** Control response to 200 msec 650 nm flash. **B:** 30-minute ionophoresis from intracellular pipettes filled with 0.5 M cAMP creates a light-adapted phenotype in the injected cell.

Fig. 6. Simultaneously recorded HC pair. Stimulation with a sinusoidal 650 nm 1–15 Hz flicker ramp at two intensities differing by 1.8 log units. The control cell responded well to the dim red flicker and became saturated with the bright red flicker. On the contrary, the cAMP-injected cell was relatively insensitive to the dim flicker, responding vigorously to the bright flicker. Note that the injected cell, unlike the control cell, responded from the middle of its dynamic range.

Fig. 7.

Effect of dopamine on L-HC responses to kainate. Isolated HC was whole cell-clamped at -60 mV. **Top:** Control. A 300-msec puff of $50\text{ }\mu\text{M}$ kainate elicited an inward current. The slow decay of the current is due to the absence of saline wash. A 1.3-second puff of $0.5\text{ }\mu\text{M}$ dopamine was applied between the two top traces, and the test pulse of kainate was applied each 30 seconds thereafter. At 1 and 2 minutes, the KA-induced current was larger than control, but recovered to control amplitude by 4 minutes.

Fig. 8.

Flicker responses of a mesopic HC to sinusoidal stimulus of 660 nm light. **Top:** HC exposed to steady 650 nm ($\log Q=13.80$) background light. At 3–5 Hz a prominent second peak in the HC flicker response is observed (arrow). The peak disappears at 6 Hz. **Middle:** HC under a steady 527 nm background ($\log Q=12.8$). The second peak disappears under these conditions leaving only the peak with the faster kinetics. **Bottom:** The flicker stimulus.