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THE RELATION BETWEEN EXCITATION AND ADAPTATION WITHIN THE ROD'S OUTER SEGMENT

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The relation between excitation and adaptation in vertebrate rod photoreceptors was studied by measuring the membrane current of toad rod outer segments in light and darkness. Steady illumination attenuated and sped the rod response to flashes, and it suppressed membrane current noise. When steady illumination ended, the rod's electrical response, and presumably the internal transmitter which caused it, decayed quickly, but adaptation's effects persisted temporarily. This new observation suggests that excitation and adaptation are loosely coupled phenomena which may be mediated by different chemical signals.

Light causes two effects within vertebrate photoreceptors. First it causes excitation, the processes which generates the electrical signal which the photoreceptor transmits to other visual cells [24, 28]. Second it causes adaptation, a process which modifies and regulates excitation [7, 11, 12, 14, 15]. It was once believed that excitation and adaptation resulted from very different physiological mechanisms. Early evidence indicates that vertebrate photoreceptors were nearly linear transducers of the light they absorbed and that their responsiveness was not directly affected by light adaptation [21, 25–27]. Adaptation, by contrast, was produced by large networks of proximal neurons that modulated the excitatory signal as it propagated throughout the visual system [13, 26, 27]. Although such secondary neural adaptation pools exist, their importance has been diminished by newer findings which show that vertebrate photoreceptors do indeed adapt to light [4, 11, 19, 23]. Using extracellular techniques, for example, Boynton and Whitten [8] demonstrated that the sensitivity of primate cones is directly altered by steady illumination. And, using intracellular recording, Fain [14] showed that steady illumination alters the intracellular response of toad rods. Although such studies proved that adaptation affects the photoreceptors directly, they fell short of proving

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that photoreceptor adaptation is caused by the same physiological process which produces excitation. There was evidence, for example, that adaptation's effects could be mediated by feedback signals coming from more proximal cells [3], or that it was mediated by voltage sensitive mechanisms within the photoreceptor's inner segment [5, 6, 10]. Recently, however, it has become possible to measure the photocurrent which single rod outer segments produce when they respond to light [7, 17]. Since this photocurrent is the primary electrical signal which the receptor produces, the observation that it, too, is subject to adaptation strongly suggests that excitation and adaptation manifest two different effects of a single physiological process [7, 17, 29, 30]. New studies reported here show, however, that there are important differences in the time course of the excitatory and adaptation effects which occur within the rod outer segment. These differences suggest that adaptation is not controlled electrically but may, instead, be regulated by a different chemical transmitter than the one which produces the outer segment's photocurrent.

The membrane current of single rod outer segments was measured using procedures similar to Baylor, Lamb and Yau's [7, 29]. Toad (*Bufo marinus*) eyes were hemisected in dim red light, and their retinas were removed in a Ringer comprising (in mM): NaCl, 111; $MgCl_2$, 1.6; KCl, 1.5; $CaCl_2$, 1.0; D-glucose, 10; HEPES, 3, buffered to pH 7.8 with NaOH. Retinas were chopped into fragments which were transferred to an oxygenated, Ringer-filled chamber on the stage of an inverted compound microscope. Under visual control single outer segments were drawn into the barrel of a pipette, and their membrane current was measured with a current-to-voltage amplifier. The recording bandwidth was DC to 15 Hz. Flashes and steps of light were generated by calibrated light-emitting diodes.

Other investigators have found that adaptation alters the outer segment's photocurrent in three ways [7, 17, 29, 30], each of which is shown in Fig. 1. First, adaptation reduces the response to flashes, so that a smaller response is elicited when the outer segment is light adapted that when it is not (Fig. 1A). Second, adaptation reduces electrical noise which is present in the membrane current of dark adapted outer segments (Fig. 1B). Finally, adaptation speeds the photocurrent's kinetics so that a light adapted response peaks and decays faster than an equal-amplitude, dark-adapted one (Fig. 1C).

This evidence is compatible with the hypothesis that photocurrent and adaptation are both controlled by a single physiological mechanism within the outer segment. One possibility is that adaptation is directly regulated by the photocurrent itself. This hypothesis is plausible since many nerve cells are known to contain electrically excitable processes that regulate their activity. Alternately, adaptation could be controlled by the direct effects of the chemical which, according to the internal transmitter theory, produces the photocurrent. According to this widely accepted theory [4–6, 9, 26], an internal transmitter, which is produced by the rod disks when they absorb photons, quickly diffuses to the outer segment's plasma membrane, where it blocks sodium channels and produces photocurrent. This process

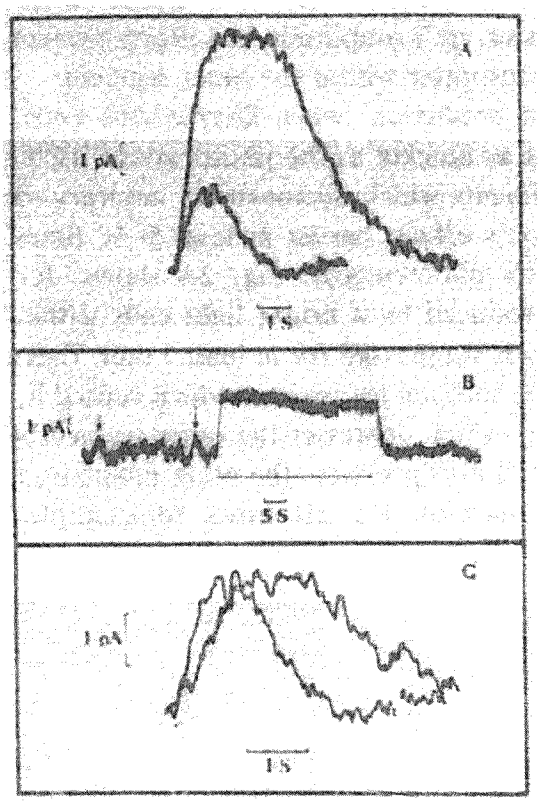

Fig. 1. Light affects the outer segment's membrane current in three ways. A: light reduces sensitivity. A flash (132 quanta/sec/μm^2) produced a larger response in darkness (upper record) than it did in light (4.59 quanta/sec/μm^2). The measured photocurrent is proportional to the length of outer segment in the electrode [7]. In this case, the full length of the outer segment was in the electrode, and the cell produced a maximum response (R_{max}) of 15.2 pA. A light (i_o) of 9.4 photons/μm^2 produced a half-maximal response. The adapting light produced a steady-state current of 4.6 pA. B: light suppresses slow, large amplitude bumps (arrows) such as others have attributed to the spontaneous isomerization of photopigment [30]. This noise is absent during illumination (1260 quanta/sec/μm^2). Only the tip of the outer segment (approximately 7–8 μm) was drawn into the electrode during this record. After the entire outer segment was drawn into the pipette, however, R_{max} = 19.2 pA and i_o = 8.4 quanta/μm^2 for this cell. C: light speeds the response to flashes. A flash (132 quanta/sec/μm^2) which occurred in the presence of an adapting light (4.59 quanta/sec/μm^2) produced a current (unbroken line) that peaked sooner than the equal-amplitude current (circles) produced by a flash (12.9 quanta/sec/μm^2) that occurred in darkness. Same cell as in A. Since adapting lights produced maintained currents, the baselines of the records of A and C were artificially aligned for comparison. Stimuli, produced by 560 nm light emitting diodes, are represented by horizontal bars. Flashes lasted 100 msec. The number of quanta absorbed by the outer segment can be estimated in the following manner. The rod photopigment absorbs maximally at 500 nm [7], and its action spectrum is described by a conventional nomogram [7]. Thus, if one assumes that the outer segment's effective collecting area is 5.1 μm^2 for non-polarized light [7], then the equivalent number of 500 nm photons absorbed during a 100 msec flash is equal to 0.17 times the flash intensity (in 560 nm quanta/sec/μm^2).

apparently occurs so quickly that the photocurrent's amplitude is a nearly perfect indicator of the concentration of internal transmitter within the outer segment.

These two hypotheses lead to a common prediction: when illumination ends, adaptation's effects should dissipate at least as quickly as the photocurrent itself. Fig. 2 summarizes the results of new experiments which demonstrate, contrary to these hypotheses' prediction, that adaptation's effects persist at least 5–10 times longer in darkness than the outer segment's photocurrent. Fig. 2A shows, for example, that although the photocurrent produced by a bright light ends within about 10 sec in darkness, the flash sensitivity is suppressed for at least 1 min. Even though the photocurrent, and presumably the internal transmitter which caused it, ends quickly, some adaptation signal persists which depresses the outer segment's response to illumination. This persisting signal also produces the other adaptation effects which are manifest during steady illumination. Fig. 2B shows, for example,

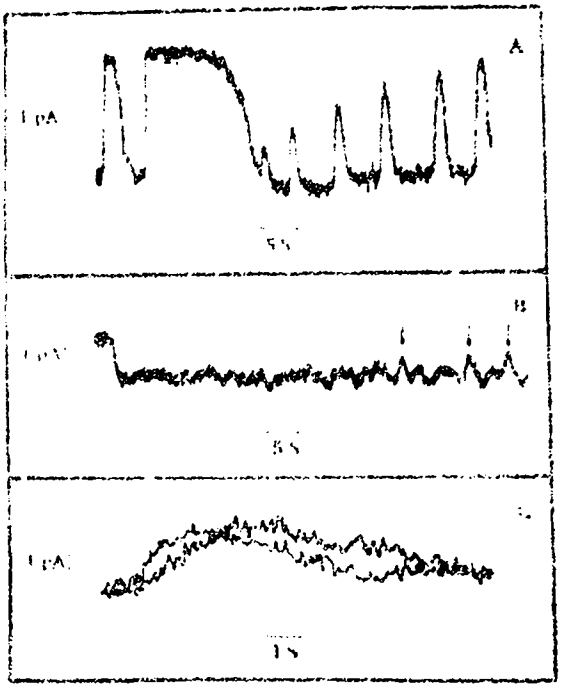

Fig. 2. Adaptation's effects persist in darkness after the membrane current had ended. A: a flash (190 quanta/sec/μm^2) which produced a large response in darkness, produced smaller responses following an adapting light (209 quanta/sec/μm^2). After the adapting light was extinguished, flash-sensitivity was temporarily depressed even though the membrane current had ended. During this recording approximately one-half of the outer segments was drawn into the electrode. Under these conditions R_{max} = 8.4 pA and i_0 = 6.1 quanta/μm^2. B: a 20-sec adapting light (1260 quanta/sec/μm^2) ended as the record began. Although the membrane current ended quickly, electrical noise (arrows) did not reappear immediately. Noise suppression following adaptation is also evident in Fig. 1B. Same cell as in Fig. 1B. C: a 30 sec adapting light (3.6 log quanta/sec/μm^2) was extinguished. Sixty seconds after the membrane current had ended, a flash (80 quanta/sec/μm^2) produced a current which peaked sooner (unbroken line) than an equal-amplitude current produced two minutes later by another flash (9.28 quanta/sec/μm^2). For this cell R_{max} = 25.4 pA and i_0 is 6.36 quanta/μm^2.

that noisiness in the membrane current is suppressed in darkness long after the photocurrent has ended, and Fig. 2C shows that the flash response elicited in darkness after the photocurrent's end, peaks and decays sooner than an equal-amplitude response elicited after the outer segment has dark-adapted. Thus, each of the adaptation effects which occurs during steady illumination persists in darkness after excitation has ended.

One interpretation of these effects is that internal transmitter desensitizes the outer segment's membrane just as other chemical transmitters densensitize their postsynaptic targets [18]. In both cases the reductions in sensitivity persist after the transmitter has been removed. Despite this and other similarities between adaptation and desensitization, however, there is an important difference between these two effects which underscores an interesting property of outer segment adaptation. Although desensitization produces a refractoriness in the postsynaptic cell so that, in the limiting case, additional transmitter has no effect, adaptation alters the sensitivity of the outer segment without actually causing the membrane to become refractory. Thus, an outer segment which can produce a 20 pA current in darkness can also produce a 20 pA current in light, but in the latter case a portion of the photocurrent will represent the cell's response to the adapting light. Adaptation does not diminish the outer segment's capacity to produce photocurrent. Rather, it adjusts the cell's sensitivity so that brighter lights are needed to produce an incremental response.

Since adaptation does not limit the photocurrent which the outer segment can produce, it must not actually inactivate the receptor sites within the membrane, as desensitization apparently does. Instead, adaptation probably diminishes the amount of internal transmitter which is released by the disks when they absorb photons. The precise basis for this effect is unknown, but it is plausible, as others have suggested [1], that adaptation's effects within the outer segment are mediated by a second form of internal transmitter, which spreads among the disks and alters their production and release of internal transmitter. The present result, that adaptation persists longer in darkness than excitation, could then be explained by postulating that the adaptation transmitter survives longer in darkness than the internal transmitter which produces photocurrent.

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