Blue-sensitive cones of the cat produce a rodlike electroretinogram

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Two cone mechanisms are identifiable in the strongly yellow light-adapted electroretinogram (ERG) of the arterially perfused cat eye. One has its maximum spectral sensitivity near 555 nm; the other has its maximum near 450 nm. The former cone system produces a much larger signal with characteristics of a typical cone or inhibitory ERG. The latter cone system produces a small, saturable signal (less than 5 µV) which resembles a rodlike or excitatory ERG. The results imply that the latter ERG is generated by blue-sensitive cones, which form a small fraction of the total cone population and share some physiological and perhaps anatomical properties of rods.

Key words: blue-sensitive cones, cat electroretinogram, off-effect, arterially perfused eye

Cats, like many other mammals, have, in addition to cone mechanisms sensitive to long or middle wavelength, a short wavelength—or blue-sensitive mechanism. In cat this blue mechanism has been detected in the responses of single neurons in the retina1-2 and lateral geniculate nucleus3 as well as in the ERG,4 the latter being obtained with flickering stimuli of relatively high frequency. By using selective chromatic adaptation, directly coupled (d.c.) recording, and electronic averaging techniques, we have identified the low-frequency waveform of the isolated blue-sensitive cone ERG of the cat. When so isolated, this response, in contrast to the ERG of the middle wavelength-sensitive cones, resembles the E (excitatory) or rod ERG rather than the I (inhibitory) one, so characteristic of cones.5 A brief abstract of these results has been published.

Materials and methods

The isolated arterially perfused cat eye preparation7-9 was used in order to facilitate d.c. stability. Adult cat eyes were enucleated and perfused through the ophthalmic artery (2 to 4 ml/min) with Eagle’s tissue culture medium containing 25% newborn calf serum, equilibrated with 95% oxygen and 5% CO₂. It was kept in a heated closed chamber with its temperature maintained at 37° C. The d.c. ERG was recorded by chlorided silver electrodes, one contacting the cornea and the other the sclera at the posterior pole of the globe through saline-moistened cotton wicks. The signal was amplified by a Tektronix 26 A2 amplifier, summed by a Nicolet 1060 computer (n = 16 or 32) and recorded on an X-Y plotter. A 1000 watt high-pressure Xenon arc lamp provided two light beams, one a steady adapting beam and the other a test beam which was pulsed for 500 ms with a frequency of 50 Hz. The light beam was focused on the fundus of the eye through a rubber optic cone with a diameter of 10 mm.
Fig. 1. Averaged (n = 32) ERG d.c. recordings from the isolated arterially perfused cat eye preparation in response to monochromatic full-field light flashes of 444, 511, and 583 nm during exposure to an intense steady yellow background (100,000-fold above rod b-wave threshold). The stimulus strength is shown beside each record (log₁₀ quanta/sec/μm²; stimulus duration (500 msec) is indicated on the lowermost record, calibration on lower left.

Results

Fig. 1 shows ERG responses produced by blue (444 nm), green (511 nm), and orange-yellow (583 nm) stimuli superimposed on a steady yellow background, which strongly light-adapted rods (equivalent to 9.2 × 10⁵ quanta/sec/μm² at 503 nm) as well as the middle wavelength-sensitive cones (equivalent to 1.5 × 10⁶ quanta/sec/μm² at 546 nm). The rod b-wave threshold of 9.2 quanta/sec/μm² was about 3 log units above the rod threshold reported in cat ganglion cells¹¹ (6 × 10⁻³ quanta/sec/μm²); this agrees well with the fact that the human b-wave threshold is about 3 log units above subjective sensory threshold.¹² ¹³ The 444 nm stimulus elicited a b-wave (peak latency = 45 to 60 msec), followed by a slow negative response; a small a-wave preceded the b-wave; at the offset of the stimulus another slow negative response appeared but no positive d-wave was seen at any intensity level. The 583 nm stimulus elicited a quicker b-wave (peak latency = 25 to 40 msec), followed by a slow negative response; a relatively large a-wave preceded the b-wave; at the offset of the stimulus a conspicuous d-wave appeared followed by a slow negative response. Light of the middle of the spectrum (511 nm stimulus) elicited quick b- and d-waves, which were considerably
Fig. 2. Action spectra obtained from V-log Int functions by means of constant-response criteria. A, ERG on-responses. Filled circles, Negative on-responses; open circles, positive on-responses (b-wave). B, ERG off-responses. Filled triangles, Negative off-responses; open triangles, positive off-responses (d-wave). Vertical bars reflect standard deviations calculated from 10 experiments. The solid lines represent an unweighted addition (S + L) or subtraction (S — L, in absolute values) of a 455 and 555 nm Dartnall nomogram (S and L, respectively). Interrupted line indicates the missing short-wavelength mechanism in the positive off-effect. Data are corrected for preretinal absorption loss; sensitivity is given in reciprocal values as \( \log_{10} \text{quanta/sec/}\mu\text{m}^2 \).

smaller than those produced by the longer wavelength stimulus (583 nm); it produced large negative responses at both the onset and offset of the stimulus.

The spectral sensitivities of these responses are shown in Fig. 2. The action spectra of the slow negative on- and off-responses (filled symbols) were broad functions, which could be approximated by the unweighted addition of two Dartnall nomograms (solid lines, S + L), one peaking at 455 nm (S) and the other at 555 nm (L). The action spectra of the b- and d-waves showed a different pattern. The spectrum of the b-wave (open circles) had two peaks near 450 nm and the other near 560 nm, with a minimum near 500 nm. This spectrum could be better approximated by the subtraction rather than the addition of the previous two nomograms (solid lines, S — L, absolute values). The action spectrum of the d-wave (open triangles) had only one peak at 560 nm and could be matched by a single nomogram with its maximum at 560 nm (solid line, L); it showed no indication of a blue mechanism (interrupted line).

Evidence for two different photoreceptor mechanisms contributing to this yellow light-adapted ERG was also apparent in the V-log Int functions of the b-wave (Fig. 3). Increasing intensities of short wavelength stimulation (dots), which at relatively dim light levels affected only the 450 nm mechanism, produced a slight gradual increase in b-wave amplitude and, at about 0.5 \( \mu \text{V} \) reached saturation. Saturation amplitudes of this blue cone response ranged from 0.3 to 5 \( \mu \text{V} \). Amplitudes of the b-wave obtained with long-wavelength stimulation, which affected only the 560 nm mechanism, increased steeply with increasing intensities of stimulation (triangles) and failed to saturate even with our strongest long-wavelength stimulus. Therefore, it was impossible to
match V-log Int functions obtained with long-wavelength stimuli to those obtained with short wavelengths by compensating for effective energy differences, i.e., sliding the curves along the intensity axis. If the V-log Int function obtained with dim, short-wavelength stimuli was multiplied by a factor of about 10 (Fig. 3, dashed line), it was possible to approximate the steepness of the slope of the function obtained with long wavelengths; this factor may be related to the ratio of short- to middle-wavelength cones in cat retina.

The study was repeated in the presence of a blue-adapting field, which had an approximately equivalent effect on the rods as the yellow light. In this case, all wavelengths produced identical ERGs when compensated for effective energy, which closely resembled those obtained with long-wavelength stimuli on a yellow background (Fig. 1, 583 nm response).

Discussion

The results indicate that at least two photoreceptor mechanisms contribute to the cat's ERG adapted by a strong yellow light. This is especially apparent in the quick positive components of the response, occurring at the onset and offset of a light stimulus, the b- and d-waves, respectively. One mechanism with an action spectrum that has its maximum near 450 nm generates a comparatively slow b-wave which saturates at low response levels and produces no d-wave. This response is considered to reflect the activity of blue-sensitive cones. The possibility that rods produce this response seems unlikely. It is detected on background lights which are approximately 20 times stronger than the saturation value of the rod mechanism of the cat; it resembles the behavior of the primate blue cone ERG in being best detectable with relatively weak short wavelength stimuli before saturation of the response occurs and it is then swamped by the b-wave of the much more predominant longer wavelength sensitive cone system; it has an action spectrum with a peak at about 450 nm, well to the short wavelength side of the maximum sensitivity of a rod mechanism. Opponent interactions could shift action spectra away from absorption spectra, but they would have to be extremely strong to do so. Opponent interaction between rods and cones does not seem to occur in the b-wave of the ERG.

In cat retina, rod-cone interactions either at the receptor or horizontal cell level, sites at which opponent interactions observable in the b-wave might be expected, are synergistic and not antagonistic. The extremely low sensitivity of the yellow light-adapted b-wave in the range of 500 nm (Fig. 2, A) suggests that some opponency may exist between the blue and the longer wavelength-sensitive cones contributing to this response. Perhaps opponency would be directly detectable if these two cone mechanisms were made to interact in double-pulse experiments. It is interesting that the a-wave
of the ERG, which presumably represents a more peripheral retinal event than the b-wave, shows no indication of cone opponency.

The middle-wavelength cone mechanism which essentially dominates the photopic ERG of the cat produces a response which has all the characteristics of a classic cone ERG, the so-called I or inhibitory ERG of Granit, large and quick a- and b-waves and a positive response or d-wave at the termination of a light pulse. The short-wavelength or blue-sensitive cone mechanism which makes an extremely small contribution to the cat’s ERG produces a more rodlike, so-called E or excitatory, ERG: a small negative initial response, a slow b-wave, and only a negative response without a d-wave at the termination of a light stimulus.

The electrophysiological similarities between the ERG of the blue cones and rods may reflect their retinal circuitry. Perhaps blue cones share some of the unique anatomical characteristics of rods. In cat, there are two bipolar systems for cones, the invaginating and the flat varieties which are involved in the on- and off-center channels of the retina, respectively. The rod system has only one anatomical class of bipolars and an unusual pathway to ganglion cells via an intermediary or AII amacrine cell. The circuitry of the blue cone system is unknown, but perhaps, like rods, it has only one class of bipolars which may be responsible for the absence of a positive off-response in its ERG. The possibility that the blue-sensitive cone mechanism acts through only one bipolar system may also be relevant to the observations that blue cones usually mediate on-center channels in both the cat and monkey visual system.

The fact that blue cones may be more rod-like than other cones is not surprising in view of the many unusual properties this system exhibits in human vision. Blue cones, like rods, have a low spatial resolution and a relatively low saturation level. Genetically, the blue cone system does not obviously follow the classic sex-linked inheritance pattern of the red-green system, although our understanding of this problem is still unclear. Diseases of the external retinal layers have been traditionally considered to have the tendency to produce an acquired form of tritanopia, whereas acquired protan-deutan defects are usually more characteristic of inner layer or optic nerve disease, i.e., Köllner’s rule, a characteristic which may also reflect a similarity between rods and blue cones, since the rod system, too, is relatively vulnerable to external retinal layer degeneration.

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REFERENCES


