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Reexamination of Spectral Mechanisms in the Rat (*Rattus norvegicus*)

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The spectral mechanisms of the rat were studied in electrophysiological and behavioral experiments. The photopic electroretinogram (ERG) was recorded (a) to rapidly flickering lights, (b) during the cone phase of dark adaptation, and (c) with a flicker photometric procedure. Each procedure indicated the presence of a single cone mechanism having a λ_{max} of about 510 nm. Increment-threshold spectral sensitivity functions measured in a behavioral test situation yielded the same conclusion. A behavioral experiment failed to produce any evidence for the presence of color vision in this rodent. The consistent conclusion from both behavioral and electrophysiological experiments is that the rat retina contains only a single photopic spectral mechanism.

Although the rat has a rod-dominated retina, that structure also contains a population of photoreceptors having numerous morphological features typically associated with cones (Dowling, 1967; LaVail, 1976; Sidman, 1958; Sokol, 1970). It is likewise apparent that two functionally distinct mechanisms contribute to the rat electroretinogram (ERG): There are two plateaus in the ERG flicker fusion frequency versus log intensity curve, and the ERG increment-threshold function shows a Purkinje shift (Dodt & Echte, 1961; Green, 1971). A natural conclusion is that the two classes of photoreceptors underlie the two visual mechanisms.

The ERG has been used to infer the photopigments present in the rat retina. The scotopic spectral sensitivity function is closely fit by the absorption function for a typical mammalian rod pigment having a λ_{max} close to 500 nm (Cicerone, 1976; Dodt & Echte, 1961; Green, 1971; Sokol, 1970). The photopigments responsible for the photopic mechanism are still in question. Several experimenters have noted features of the photopic ERG consistent with the presence of a pigment peaking at a wavelength longer than 500 nm (Dodt & Echte, 1961; Ernst & Kemp, 1975; Green, 1971; Pearlman, 1978). On the other hand, Dowling (1967) measured the photopic mechanism to have the same spectral sensitivity as the scotopic mechanism and suggested that the two mechanisms might utilize the same photopigment. The most explicit measurements are those of Cicerone (1976), who detected three spectral mechanisms in the ERG having peaks at 450, 520, and 560 nm. The photopic mechanism makes only a weak contribution to the rat ERG, so the variations in results in these experiments are perhaps not surprising.

A second approach to the question of spectral mechanisms in the rat involves measurements of behavioral discriminations. During the 1930s, a number of studies were run to determine whether the rat has color vision. Although some experimenters reached a negative conclusion (Coleman & Hamilton, 1933), others (Walton & Bornemeier, 1939) found evidence that rats can perform color discriminations and thus have multiple spectral mechanisms. A more recent measurement of increment-threshold spectral sensitivity in the rat also suggests the presence of two photopically active spectral mechanisms (Birch & Jacobs, 1975).

In light of the immense number of experiments in which experimenters have required rats to make visual discriminations, and of the contemporary utilization of this animal for numerous studies of light-induced retinal degenerations (Williams & Baker, 1980), it is remarkable that a fundamental feature of the visual system of the rat remains poorly understood. We have tried to remedy this deficiency by reexamining spectral mechanisms in the rat in a parallel series of ERG and behavioral experiments. The consistent conclusion is that the rat possesses only a single photopic spectral mechanism. That mechanism has a peak sensitivity at about 510 nm.

ERG Measurements

The ERG has been routinely used as a tool for eliciting photopic responses from the visual system (for a review, see Armington, 1974). We employed three different procedures to measure the spectral sensitivity of the rat ERG under conditons that disadvantage the rods: determination of spectral sensitivity to fast flickering lights, spectral sensitivity measured with the technique of flicker photometry, and measurement of spectral sensitivity during the cone phase of dark adaptation.

Method

Subjects

The subjects were young adult pigmented rats (Long-Evans and Zucker strains). Both sexes were used. Animals were housed under standard colony conditions which included constant temperature/

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humidity and a 12:12 hr cyclic light regimen (mean cage illuminance = 13 lx).

Apparatus

A three-beam optical system was used. One beam originated from a Bausch and Lomb high-intensity grating monochromator (half energy passband = 10 nm). The source was a tungsten-halide lamp (100 W, 12 V); its intensity was controlled with a circular neutral density wedge. The other two beams came from tungsten-halide lamps (50 W, 12 V). The content of these beams was controlled by using neutral density step filters and interference filters (Ditric, half-energy passband = 10 nm). All three of the lamps were underrun at 11 V from a regulated dc power supply. Each of the beams contained a high-speed electromagnetic shutter (Uniblitz, Vincent Associates). These shutters were driven by a programmable digital timer. The three beams were optically superimposed and focused on a final lens so that they illuminated a circular 53° patch of the retina in Maxwellian view. Light calibrations were made with a PIN 10 DF silicon photodiode (United Detector Technology).

The ERG was recorded with a silver ring electrode placed against the cornea. The reference electrode was sewn into the skin above the eyelid, and the ground electrode was placed against the inside of the cheek. For recording the responses to single light flashes and to flickering lights, ERGs were differentially amplified (bandpass = 0.2–1000 Hz) and averaged with an Ortec 4623 Averager. These averaged responses were measured from a calibrated oscilloscopic display. The signal processing for the flicker photometric measurements was different. That technique has been described in detail elsewhere (Neitz & Jacobs, 1984). In that case, as in classical flicker photometry, responses to an alternating train of light flashes from two sources (a test and a reference light) are compared. The alternation of these two lights was separated by a dark interval equal in duration to that of each light flash. In order to accomplish the photometric equation, the differentially amplified ERG responses from the two lights were fed through active narrow bandpass filters (half-voltage bandpass = 0.2 × the center frequency). The center frequency of the filter was set to equal the stimulus frequency. When the intensities of the test and reference light are greatly disparate, the filter output is sinusoidal. Reversal of the relative intensities causes a 180° phase shift in the output waveform. At an intermediate intensity, the amplitude of the response is minimized, and the phase is intermediate. That setting defines a photometric match between the two light sources.

Procedure

Rats were anesthetized with ip injections of sodium pentobarbital and chloral hydrate. The pupil of the test eye was dilated by topical application of atropine sulfate, the animal was placed in a stereotaxic instrument, and the electrode was installed. Normal body temperature was maintained through the use of a circulating water heating pad.

Flicker spectral sensitivity. The stimuli were obtained from the monochromator beam. Flickering stimuli (light/dark ratio = 1) were presented for 1 s before recording began. After that, responses to at least 80 successive flashes were averaged for each stimulus condition. Responses were recorded to monochromatic stimuli from 440 to 620 nm (usually in steps of 20 nm). At each test wavelength, a minimum of four different stimulus intensities spaced at intervals of 0.3 log unit were presented. All stimulus combinations were run at least twice, with the two series completed in reverse spectral orders. Responses to a reference test light were obtained periodically during recording session to assure that no systematic changes in sensitivity had occurred.

Spectral sensitivity during cone dark adaptation. In this procedure responses to monochromatic test flashes (200 ms) were recorded shortly after the presentation of an achromatic flash (200 ms). The time between the offset of the adapting flash and the test flash was 400 ms. Responses to a minimum of 20 test flashes were averaged for each stimulus condition. Measurements of spectral sensitivity were made with the same general procedure as described above. The effectiveness of the adapting flash on the rat retina was computed by using the measurements of the optical characteristics of the rat eye and estimates of the receptor density in this retina provided by Cone (1963). According to these estimates, the adapting light yielded 7.68 log quanta/receptor/second. According to these same assumptions (Cone, 1963), each such flash would be expected to bleach 30% of the rod photopigment in the area of the retina illuminated.

Flicker photometry. As described in Results, several different flicker rates were examined in this experiment. In each case the reference light was achromatic and had a corneal radiance of 33 μW. The test light was obtained from the monochromator. To make a sensitivity measurement, we first set the density wedge in the monochromator beam to an arbitrary position. A train of 100 stimulus cycles (each consisting of test light, dark interval, reference light, dark interval) was presented, and the responses to the last 60 of these were averaged. The phase and amplitude of the response was used to guide the subsequent change in wedge setting needed to better null the response to the reference light. This procedure was done iteratively until the best null position was determined. Sensitivity measurements were made for monochromator settings between 620 and 440 nm in steps of 10 nm.

The spectral sensitivity values obtained in each of the three experiments were compared with wavelength-dependent visual pigment nomograms (Ebrey & Honig, 1977) using the polynomial expressions derived by Dawis (1981). A computer was used to determine the spectral peak (to the nearest nanometer) of the nomogram that gave the best fit to the sensitivity values. To accomplish this, we computed the sum of the differences squared between each sensitivity value and the nomogram for each of the possible nomograms. The nomogram providing the smallest average difference squared (i.e., the estimate of goodness of fit) was selected as best fitting the data.

Results

As noted above, a Purkinje shift has been frequently detected in the rat ERG. We first established that such a shift could be seen under the conditions of this experiment. ERG sensitivity was measured for two monochromatic test stimuli (500 and 600 nm) at flicker rates ranging from 5 to 30 Hz. A typical result obtained from this procedure is shown in Figure 1 which presents the differences in sensitivity to these two lights as a function of flicker frequency. The resulting function shows the change in spectral sensitivity characteristic of a Purkinje shift. The function has two plateaus; at low flicker rates the difference in sensitivity to the two test stimuli is about 1.3 log units. That value is close to the difference predicted on the basis of the operation of rat rhodopsin (Bridges, 1959), and thus at the low flicker rates the usual rat scotopic mechanism can be seen. A sharp break in the function occurs between 10 and 15 Hz, and above those rates the 500/600 sensitivity difference again becomes asymptotic at about 0.9 log unit. Under these conditions, therefore, flicker rates above about 15 Hz elicit responses from a photopic mechanism.

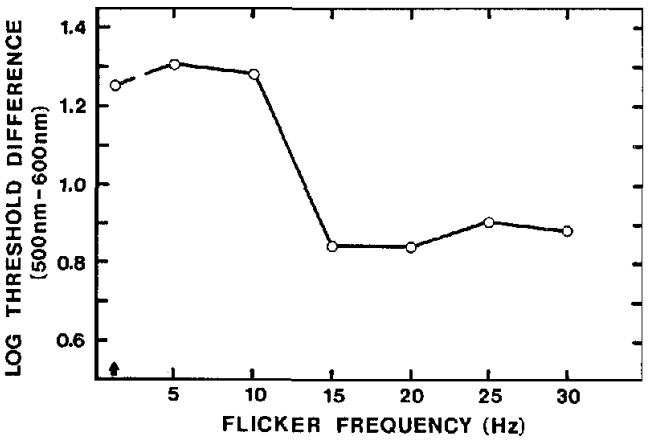

Figure 1. Electroretinographic sensitivity differences to 500-nm and 600-nm lights flickering at the indicated frequencies. (Each circle represents the difference in intensity required to produce a criterion 20-μV response [trough-to-peak amplitude] at the two test wavelengths. The circle to the left [above the arrow] shows the difference obtained for single 200-ms light flashes.)

Complete spectral sensitivity functions were determined with 25-Hz flicker, a value that Figure 1 indicates should yield responses from the photopic system. The results obtained from 4 animals are shown in Figure 2. Individual sensitivity values (solid circles) represent the reciprocal of that quantal intensity required to produce 7.5-μV ERGs (peak-to-trough amplitude) at each test wavelength. The functions for the 4 animals are arbitrarily positioned on the sensitivity axis. The solid lines are the best fitting visual pigment nomograms

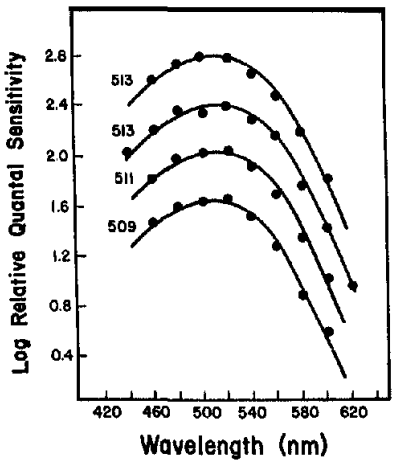

Figure 2. Flicker electroretinographic spectral sensitivity functions for 4 rats. (The plotted points are the sensitivity values for individual animals. In order to obtain these, intensity/response functions were derived for each test wavelength by plotting the amplitude of the response to 25-Hz flicker versus stimulus intensity. The sensitivity values are the inverse of the intensities required to produce responses of 7.5-μV amplitude. The number to the left of each function is the λ_{max} of the best fitting nomogram photopigment [solid curves]. The four curves have been arbitrarily positioned on the sensitivity axis.)

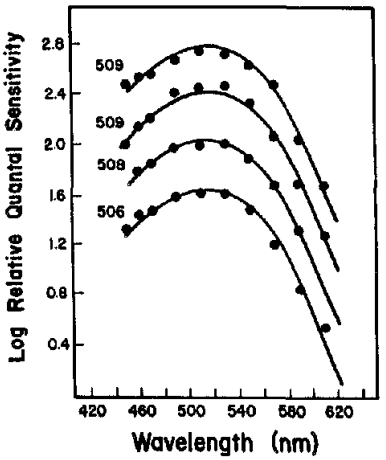

Figure 3. Photopic spectral sensitivity functions determined for 4 rats during the cone phase of dark adaptation. (Other conventions are as described for Figure 2.)

derived as described above. The value for the spectral peak of each such nomogram is indicated on the function. Two aspects of these results are noteworthy: (a) single visual pigment nomograms provide excellent fits to these ERG photopic functions for all subjects with no hint of any systematic departures from the nomogram curves; the computed goodness of fit values for the 4 animals ranged from 2.1×10^{-3} to 2.8×10^{-4} log unit, and (b) the peaks of the best fitting curves cover a relatively restricted spectral range having a mean value of 511.5 nm ($\pm$ 1.91) for 4 subjects.

Figure 3 shows the spectral sensitivity functions determined for 4 other rats with the second procedure, that is, measuring sensitivity shortly after the offset of an intense adapting flash. The individual sensitivity values are again those required to produce criterion responses of 7.5-μV amplitude (measured from the baseline to the peak of the b-wave). The other conventions in Figure 3 are the same as those described for Figure 2. It is apparent that the spectral sensitivity functions determined in this second way are also very well accounted for by curves derived from single visual pigment nomograms (goodness of fit values ranged from 1.1×10^{-3} to 8.0×10^{-4} log units). Note again the absence of any systematic departures in the data from the nomogram functions. The peaks of the best fitting functions for 4 rats in this case had a mean value of 508.0 nm ($\pm$ 1.41).

The third technique employed to measure ERG photopic spectral sensitivity in the rat, flicker photometry, proved to be the most efficient of the three procedures. As a consequence, it was possible in most cases to double the number of spectral points examined. Figure 4 shows results obtained from 4 rats tested with ERG flicker photometry. All of the conventions in that figure are the same as in the previous two experiments. With the high density of data points, it is even clearer that these spectral sensitivity functions can be very well fit by curves derived from single visual pigment nomograms (goodness of fit values ranged from 1.7×10^{-3} to 4.0×10^{-4} log units). A total of 11 functions were obtained with this technique; the functions shown at the top and the bottom

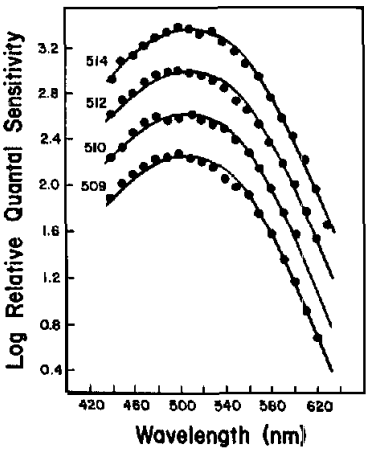

Figure 4. Electroretinographic spectral sensitivity functions obtained from 4 rats with a flicker photometric procedure. (Other conventions are as explained for Figure 2.)

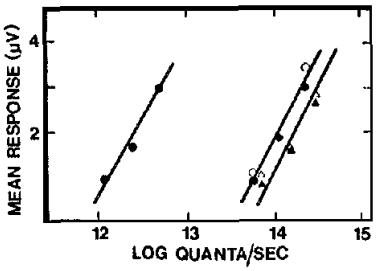

Figure 5. Effects of chromatic adaptation on the photopic electroretinogram of the rat. (The symbols indicate average response amplitudes for 25-Hz flicker. To the left is an intensity/response function obtained by using a 600-nm test light without any accessory adaptation. To the right are intensity/response functions obtained when the same eye was steadily adapted to either 450-nm, [solid symbols] or 600-nm [open symbols] lights. The test lights used were 450 nm [circles] and 600 nm [triangles]. Note that the difference in sensitivity to the two test lights does not change under the two conditions of adaptation [solid vs. open symbols].)

of Figure 4 represent, respectively, the individual functions with the longest spectral peak (514 nm) and the one with the shortest spectral peak (509 nm). The peak values for all of the remaining functions lay between these two values. For the group, the mean spectral peak was located at 510.9 nm (± 1.81).

The flicker photometric procedure was used to further explore the effects of flicker rate on derived spectral sensitivity in the rat. We found, in sum, no evidence of any systematic spectral shifts over the range from 25 to 50 Hz. Three rats tested at 25 Hz gave curves with average λ_{max} of 511.3 nm, 5 animals tested at 42 Hz had a corresponding value of 511.0 nm, and 3 rats tested at 50 Hz yielded an average peak of 510.3 nm. This result supports the conclusion suggested in Figure 1—that in the rat the ERG spectral sensitivity shift that is correlated with flicker rate is complete when the rate equals or exceeds 25 Hz.

All of the spectral sensitivity functions shown in Figures 2–4 are closely fit by curves derived from visual pigment nomograms. This suggests that the rat photopic ERG might be generated by a single spectral mechanism. If so, the form of the photopic spectral sensitivity function should not be affected by any further changes in the adaptation state of the eye. We tested this by assessing the effects of chromatic adaptation for each of the three paradigms used for measuring the ERG under photopic conditions. Figure 5 illustrates one such experiment. Shown there are intensity/response functions recorded for 25-Hz flicker. As demonstrated above, light flickering at this rate produces a photopic spectral sensitivity function. At the left in the figure are the average amplitudes recorded to a 600-nm test light from an otherwise unadapted eye. The eye was then continuously exposed to intense chromatic adapting lights, and intensity/response functions were redetermined for two test lights, 450 and 600 nm, each of which was also flickering at 25 Hz. Each of these functions was determined in the presence of both 450- and 600-nm adaptation. The assumption was that if only a single spectral mechanism contributes to the 25-Hz response, then the relative sensitivity of the eye to these two test lights should be the same under both conditions of chromatic adaptation. That this was the outcome is shown by the fact that the difference in sensitivity to the 450- and 600-nm test lights was the same irrespective of whether the eye was concurrently exposed to intense 450-nm light or intense 600-nm light.[1] Additional animals, different combinations of test and adaptation lights (440 and 460 nm were also used as adaptation lights), and utilization of the other two techniques for eliciting photopic signals all yielded results identical to those illustrated in Figure 5. We found no evidence whatsoever of differential chromatic adaptation effects in the rat photopic ERG.

Discussion

The results of these experiments paint a simple picture of the spectral mechanisms contributing to the rat ERG. In addition to rods, which produce the strong scotopic signal seen by everyone who has recorded a rat ERG, there is a photopic mechanism, probably cone based, with peak sensitivity in the range of 508–512 nm. The fact that the same result was obtained with three different techniques, each of which has been frequently employed to measure photopic responses, and with more than 20 individual subjects makes it hard to doubt the validity of this finding. All of the previous ERG studies listed in the introduction found a photopic mechanism in the rat peaking in the 500–520-nm range. The present experiments define the location of that mechanism more precisely.

[1] Because neither the density nor the retinal distribution of the photoreceptors underlying the rat photopic ERG is known, it is impossible to calculate directly the effects of these adapting lights on the retina. However, Figure 5 shows that the threshold for the photopic mechanism was elevated about 2 log units in the presence of these adapting lights. In humans, a cone threshold elevation of this magnitude requires a cone bleach of about 70% (Rushton, 1963). At the least, therefore, it seems apparent that the chromatic adaptation conditions employed in this experiment produced a very substantial adaptation of the rat photopic system.

In addition to specifying the location of peak sensitivity of the photopic ERG mechanism, the results of these experiments further suggest that mechanism reflects the operation of only a single class of photopigment. Our results thus do not support those of Cicerone (1976), who detected two additional mechanisms (λ_{max} = 450, 560 nm). Although the evidence for the presence of only a single photopigment type is quite strong, based as it is on both the shape of the spectral sensitivity function and on its resistance to change in the face of chromatic adaptation, it is not possible from these experiments to absolutely rule out the presence of other photopic pigments in the rat retina. It might be argued that an additional cone system is present but does not contribute to the ERG recorded under current range of stimulus conditions or that perhaps its contribution is too feeble to influence the spectral sensitivity function or to register its presence in the chromatic-adaptation experiments. To pursue these possibilities further, we conducted a second series of experiments involving measurements of behavioral discrimination capacities of rats.

Behavioral Experiments

Sperling and Harwerth (1971) showed that if photopic spectral sensitivity is measured for either humans or macaque monkeys by determining increment thresholds for chromatic stimuli presented on achromatic backgrounds, the resulting functions have three distinct peaks. The usual explanation for the appearance of these functions is that they reflect the operation of spectrally opponent neurons and thus that the peaks are produced by opponent interactions between the three classes of cone photopigment known to be present in the retinas of humans and macaques (King-Smith & Carden, 1976). Subsequently it has become clear that multiple peaks are a characteristic feature of increment-threshold spectral sensitivity functions in a wide range of species including turtles (Muntz & Sokol, 1967), squirrel monkeys (Jacobs, 1972), tree squirrels (Jacobs, 1974), ground squirrels (Jacobs, 1978), pigeons (Graf, 1979), rabbits (Nuboer & Moed, 1983), cats (Loop & Millican, 1985), and tree shrews (Jacobs & Neitz, in press) as well as the primate species listed above. It begins to appear that any species having a retina containing two or more classes of cone pigment will produce an increment-threshold spectral sensitivity function with multiple peaks. The number of such peaks and their spectral placement will depend, obviously, on the spectral properties of the underlying cone pigments and the characteristics of the neural interactions. To gain further insight into the nature of the spectral mechanisms in the rat, we measured increment-threshold spectral sensitivity functions for a variety of adaptation conditions. An attempt was also made to determine whether color vision could be demonstrated in the rat.

Method

Subjects

Three male Long-Evans rats were tested. They were food deprived for a period of 22 hr prior to each test session.

Apparatus

The apparatus was a general purpose device developed for measuring visual sensitivity and color vision in a variety of mammalian species. It has been described in detail elsewhere (Jacobs, 1983); consequently, only a brief description is provided here.

The task was a three-alternative, forced-choice discrimination in which the rat viewed three circular panels (2.5 cm in diameter) mounted in a line (center to center = 5 cm) along one wall of a test chamber. On each test trial, two of the panels were identically illuminated, and the third received a different illumination. The rat was trained to select the uniquely illuminated panel and indicate its choice by touching that panel. A correct choice was reinforced by delivery of 0.1 ml of grape juice into a receptacle located directly beneath the panel. Over trials, the location of the uniquely illuminated panel was varied. So, too, was the nature of the difference between the positive and the two negative panels. This permitted the determination of discrimination thresholds.

The stimulus panels were transilluminated by an optical system located outside the test chamber. For the experiments reported here, two different light sources were employed. One originated from a tungsten-halide lamp. Through a series of lenses and mirrors it was used to diffusely and equally illuminate each of the three stimulus panels (here called backgrounds). Spectral and intensive characteristics of this beam were controlled by inserting step filters into the beam. The other source was a grating monochromator (Instruments SA Model H-10) having a half-energy passband of 10 nm. This monochromator had a tungsten-halide lamp (24 V, 150 W). The output from the monochromator could be added to any of the stimulus panels so that it diffusely illuminated the entire panel. This constituted the test stimulus. The wavelength of the monochromator output was changed through the use of a stepping motor. The intensity of the test beam was controlled with a variable neutral density filter which was also positioned with a stepping motor. High-speed electromagnetic shutters located in the beams from both sources were employed to control the presentation of the stimuli. The stimulus panels were made of glass. The exterior (optical system) surface of the panels had a ground diffusing surface; the interior (stimulus chamber) surface of the panels was coated with a transparent layer of indium oxide. This conductive layer was connected to an electronic circuit which detected when the stimulus panel was touched.

The test chamber was sound shielded and lined with white Formica. A fluorescent lamp mounted in the ceiling was used to provide ambient illumination at an appropriate level (see below). A tone generator with a small speaker was mounted in the chamber to provide a cuing tone. A fan was used for ventilation. All of the details of stimulus selection, timing, and presentation as well as delivery of reinforcement and recording of responses were accomplished through the use of a laboratory computer.

Procedure

By means of a shaping procedure, the rats were taught to select that stimulus panel, from among the three, that was uniquely illuminated. In the initial training, they were required to discriminate bright monochromatic test lights presented on dim achromatic (4,800 K) backgrounds. The wavelength of the positive light was varied randomly between two values, 460 and 520 nm. Once the animal was correctly selecting the positive stimulus on close to 100% of the tests, the discrimination tests were initiated.

Spectral sensitivity. In order to measure spectral sensitivity, monochromatic lights were presented over a span of intensities (in steps of 0.3 log unit) sufficient to yield a performance range from near 100% correct to chance (33%). This usually required between four and six intensity steps. Monochromatic lights were tested at 20-nm

intervals from 440 or 460 nm to 600 nm. Each intensity/wavelength combination was presented in a five-trial block. The order in which the blocks were run was random through each entire spectral sequence. Subjects completed at least two such spectral runs in each daily test session.

Each stimulus presentation was signaled to the subject by the occurrence of a concurrent cuing tone. The test trial terminated when the animal responded or after 2 s without a response. The intertrial time was 4 s.

Spectral sensitivity functions were derived for each of several adaptation conditions: (a) panel luminance = 27.4 cd/m^2, chamber illuminance = 31 lx; (b) panel luminance = 0.21 cd/m^2, no chamber illuminance; (c) panel luminance = 0.86 cd/m^2, chamber illuminance = 31 lx; and (d) panel luminance = 6.85 cd/m^2, chamber illuminance = 31 lx. Spectral sensitivity was also determined under conditions of chromatic adaptation. In that case, the panels were steadily illuminated with achromatic light (7 cd/m^2); the chamber illuminance was 93 lx. Next, either 460- or 580-nm light was added to all of the panels. The intensities of these were adjusted according to behavioral criteria as described below. For each condition, testing continued until the animal had accumulated 100 test trials at each intensity/wavelength combination. From these results psychometric functions were drawn for each test wavelength. Threshold was defined as the intensity required to maintain performance at the 99% confidence level (for 100 trials this corresponds to 46% correct). Visual pigment nomograms were fit to these values by using the same procedures as for the ERG experiments.

Color vision. All 3 subjects were run on the spectral sensitivity tests. One rat was additionally examined in a test of color vision. For this subject the color vision test was run immediately following initial response shaping but prior to the subject's participation in the spectral sensitivity tests.

As in the spectral sensitivity tests, the positive stimulus (the test light) was produced by the monochromator, the two negative stimuli by the other light source. For this test, an additional shutter system was employed so that the test light replaced one of the backgrounds on each trial rather than being added to the background. All other details of trial timing and spacing were identical to those in force during the sensitivity tests.

A demonstration of the presence of color vision requires successful discrimination between equiluminant lights differing in spectral content. We attempted this demonstration in two ways. In one we sought to determine whether the rat could discriminate between 560- and 460-nm lights. The 560-nm test light was from the monochromator. The 460-nm light illuminated the two negative panels. It was obtained by passing the background light beam through an interference filter (Ditric, half-energy passband = 10 nm). The luminance of the negative panels was set to 2.0 cd/m^2. This is a value that the spectral sensitivity results (see below) indicate should be within the operating range of rat rods and cones. The intensity of the test light was varied widely around the point that was predicted (from spectral sensitivity determinations made on other rats) to be equiluminant to the 460-nm light. From that point of equality, the intensity of the 560-nm light was varied in steps of 0.1 log unit to span a range of ± 0.9 log unit from the predicted equation. These intensities were presented randomly in blocks of five trials each throughout each test session. A second attempt to establish the presence of color vision was done in the same manner except that the positive light was 470, 480, 530, or 550 nm and the negative panels were illuminated with achromatic light having a luminance of 3.4 cd/m^2. During all color vision tests, the interior of the test chamber had an average illuminance of 31 lx.

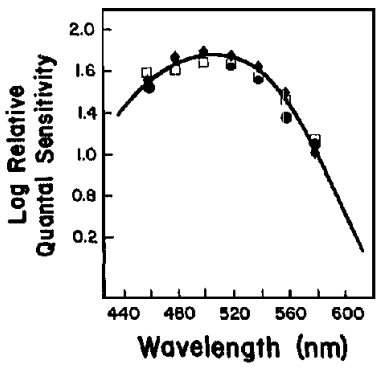

Figure 6. Increment-threshold spectral sensitivity functions obtained from 3 rats under identical adaptation conditions (background = 27.4 cd/m^2). (The values obtained from the individual animals have not been equated in any way. The solid line fit through the data points is for a nomogram photopigment having a λ_{max} of 510 nm.)

Results

Spectral Sensitivity

Figure 6 shows increment-threshold spectral sensitivity functions obtained from 3 rats under the highest level of light adaptation obtainable in the test situation. For these measurements the stimulus panels were steadily illuminated with achromatic light at a level of 27.4 cd/m^2. Three features of these photopic functions are remarkable: (a) the threshold sensitivities of the 3 subjects are closely similar with the variation across subjects, being no greater than 0.1 log unit at any of the test wavelengths, (b) these functions are simple in form with no hint of any secondary peaks or depressions, and (c) the data are well fit by the curve for a nomogram photopigment having a λ_{max} at 510 nm.

Figure 6 shows the rat photopic spectral sensitivity function as derived from increment-threshold measurements. In another series of experiments, we determined spectral sensitivity functions for lower levels of light adaptation. The results of these measurements are summarized in Figure 7. Each data point there represents the mean sensitivity for 3 rats at each test wavelength. The top function shows spectral sensitivity measured at low-light adaptation. In order to determine it, the fluorescent lamp providing illumination in the chamber was turned off, and the steady light used to illuminate the three stimulus panels was set to 0.21 cd/m^2. The resulting function is well characterized (solid line) by a nomogram curve having a peak at 498 nm. Note that this is the peak value previously determined for the rod photopigment in the rat (Bridges, 1959). The top function thus represents the scotopic spectral sensitivity function for the rat.

The function second from the top in Figure 7 was obtained in an analogous manner under conditions in which the steadily illuminated stimulus panels had a luminance of 0.86 cd/m^2. With these conditions the curve was again smooth in form, with a best fitting peak value of 504 nm. Increasing the panel luminance to a value of 6.85 cd/m^2 produced the spectral sensitivity function shown next to the bottom in Figure 7. It was again smooth in form; the best fitting curve derived from visual pigment nomograms had a peak value of 505 nm. Finally, the bottom curve is a replot in averaged form of the data first shown in Figure 6. It thus represents a spectral sensitivity function obtained in exactly the same

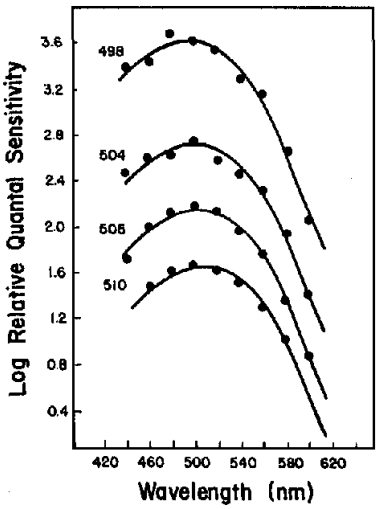

Figure 7. Rat increment-threshold spectral sensitivity functions obtained on four different backgrounds. (The plotted points on each curve represent the mean sensitivity values for 3 animals. The background luminances, from botton to top, were 27.4, 6.85, 0.86, and 0.21 cd/m^2. The values to the left are the peak values for the fitted curves.)

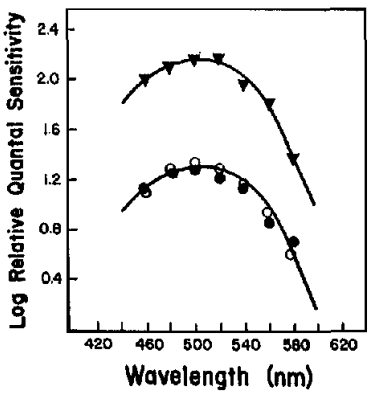

Figure 8. The effects of chromatic adaptation on rat spectral sensitivity. (The top curve [triangles] shows the mean spectral sensitivity curve for 2 rats obtained on an achromatic background [luminance = 7 cd/m^2]. At the bottom are the mean spectral sensitivity values obtained for the same 2 animals when the eye was additionally adapted to 460-nm light [filled circles] or to 580-nm adaptation [open circles]. The positioning of the two curves accurately reflects the amount of threshold elevation produced by these conditions of adaptation.)

manner as the other functions of Figure 7 except that the panel luminance was 27.4 cd/m^2.

The vertical spacing of the four functions in Figure 7 shows the actual magnitude of the threshold elevations produced by successively adapting the rats to higher light levels: From the dimmest to the brightest adaptation there is about a 2 log unit desensitization. These functions bracket the Purkinje shift in the rat. Of note is the fact that neither the scotopic function nor the photopic function (or either of the intermediate functions) shows any evidence of multiple peaks. Rather, all can be well accounted for by curves for single visual pigment nomograms.

An assessment of the effects of chromatic adaptation on rat spectral sensitivity was also made. Using an accessory panel illuminator, we first determined the panel luminance required to produce a photopic spectral sensitivity function like that in Figure 6. The top curve in Figure 8 shows the results. Given there are average sensitivity values obtained from 2 rats when the stimulus panels were steadily illuminated to a level of 7 cd/m^2 (ambient chamber illuminance = 93 lx). The fitted curve is that for a nomogram photopigment having a peak at 510 nm. This set of conditions thus yields behavior consistent with photopic spectral sensitivity in the rat. Complete functions were then redetermined, first when 460-nm light was added to all three stimulus panels and then later when 580-nm light was added to the panels. To accomplish this, we initially determined the amount of chromatic light at both adaptation wavelengths which was required to produce about equal desensitization at one test wavelength (600 nm). Complete functions were then redetermined for each of the two adaptation conditions. The results are shown as the bottom functions of Figure 8. The open circles there are the thresholds measured in the presence of 580-nm light; the solid circles, for the 460-nm adaptation. It can be seen that there are no significant differences in sensitivity at any of the test wavelengths under the two conditions of chromatic adaptation. Further, the curve drawn through these latter data is the same as that at the top of Figure 8; it is simply displaced downward by about 0.8 log unit. This experiment fails to provide any evidence that the photopic spectral sensitivity function of the rat can be altered in shape by chromatic adaptation.

Color Vision

The results from the two tests of color vision were clear and unequivocal. Although, with training, the rat learned to successfully discriminate between the test light and the negative lights for many of the test-light intensity settings, in neither of the two tests was the animal able to make the discrimination for all test-light intensities. Rather, in each case, there was one or two intensity settings at which the animal performed at chance levels; for lights brighter or dimmer than these, performance deviated from chance levels. We assume the former settings define the luminance equations between the test and negative lights. Failure to successfully discriminate these pairings means that the presence of color vision could not be demonstrated in this subject in these two tests. This failure is not likely to be due to a lack of practice at making the discriminations: The rat completed more than 25,000 test trials over a period of 10 weeks on the color vision tests.

Discussion

It was noted above that spectral sensitivity functions determined with increment-threshold procedures have been routinely found to possess multiple peaks, with intermediate regions of lowered sensitivity. All species for which such

functions have been determined are believed, on independent grounds, to have multiple spectral mechanisms. The clear lack of such features in the rat photopic spectral sensitivity function (Figure 6) provides prima facie evidence that this retina has only a single photopic mechanism. That conclusion is strongly reinforced by the discovery that neither of the two chromatic adaptation conditions (each of which produced considerable adaptation of the photopic mechanism) produced any change in either the shape or the spectral peak of the photopic function. The photopic mechanism behaves univariantly, and thus the reasonable conclusion from these behavioral measurements is that the rat photopic spectral sensitivity function arises from a single spectral mechanism having the shape of a nomogram photopigment with a peak location close to 510 nm.

The adaptation experiments summarized in Figure 7 indicate the presence of a spectral mechanism having a peak at 498 nm under dim adaptation conditions. That matches the measurement for the rat rod pigment and thus represents the scotopic spectral sensitivity function. After adaptation to bright lights, the spectral sensitivity function is shifted to a peak location of 510 nm; as argued, this reflects the presence of a single photopic mechanism. The spectral sensitivity functions measured at intermediate adaptations are then mesopic spectral sensitivity functions for the rat. The fact that the peaks of these functions are located intermediate to the scotopic and photopic peaks and that they, too, are well fitted by curves from photopigment nomograms is expected: Knowles and Dartnall (1977) showed that mixtures of two photopigments whose peaks are separated by a spectral distance similar to that of the rat scotopic and photopic functions will have (almost irrespective of the mixture proportions) spectra with a peak intermediate to the peaks of the two mixture components and the shape predicted by a single pigment curve.

Our conclusions differ from those in a previous behavioral study in which the presence of a significant elevation in the spectral sensitivity function at about 580 nm was taken to suggest the presence of two photopic spectral mechanisms (Birch & Jacobs, 1975). The evidence presented here, obtained from a long series of both behavioral and ERG studies, argues persuasively against there being two photopic mechanisms in the rat. Although we are unable to explain the results from the previous study, a reexamination of the test conditions used in that study suggests the possibility that the adaptation conditions may have allowed for the joint operation of rods and cones. If so, the two mechanisms whose actions were detected in the Birch and Jacobs study could have, in fact, been the same as those seen in the present experiment, that is, a *scotopic* and a *photopic* mechanism having λ_{max} values of about 500 and 510 nm, respectively.

The color vision test was not planned at the start of this investigation. However, evidence from both ERG and behavioral experiments that there is only a single photopic mechanism in the rat suggested that if the early claims for color vision in this rodent had merit, then that capacity would have to be based on a comparison of outputs from the only two spectral mechanisms the rat possesses, that is, rods plus the photopic mechanism. There is sufficient evidence from human color vision (e.g., Smith & Pokorny, 1977) to raise this as a distinct possibility. We could produce no evidence to support the claim for color vision in the rat. That conclusion should be interpreted cautiously, however, because a failure to demonstrate what is very likely a weak capacity is much less compelling then positive evidence for the same capacity. Nevertheless, under conditions that one might expect should be optimal for eliciting color discriminations (large stimulus fields subtending at least 45° of visual angle, maximally saturated spectral lights, large numbers of training trials), the animal showed no evidence whatsoever of successful discrimination.

Although the results from the present experiment cannot speak conclusively to the old argument about whether rats have color vision, it may be useful to note that only slight intensity mismatches (0.1–0.2 log unit) from the points of presumed equiluminance were sufficient for this rat to show some success at the discrimination. It thus remains a distinct possibility that earlier experiments that led to claims of color vision in this rodent did not contain sufficient control against the use of luminance-related cues.

General Discussion

Behavioral and electrophysiological measurements of the spectral mechanisms in the pigmented rat yield a consistent result: In addition to a robust scotopic mechanism, there is only a single photopic mechanism. Four independent measurements of this photopic mechanism show that its spectral sensitivity can be well accounted for by standard curves derived from single visual pigment nomograms. Estimates of the peak of the rat photopic spectral mechanism range from 508 nm to 511.5 nm in the four experiments; this provides an average value of 510 nm for the peak of this mechanism. If one makes the reasonable assumption that the 510-nm mechanism reflects the operation of cones, then the rat retina appears to contain a typical mammalian rod class and a single cone class.

In a recent discussion of the evolution of color vision, the suggestion was made that from a prototypical visual system based on only a single photopigment class, very likely in a retina adapted for nocturnal vision, a first evolutionary step might have been the addition of a single cone class with peak absorbance shifted to the long wavelength side of the rod pigment (Jacobs, 1981). A next step would involve the addition of a second cone type with maximum sensitivity in the short wavelengths; with the appropriate neural connections, this arrangement would yield dichromatic color vision. Although the literature of comparative color vision is woefully incomplete, it appeared until now that all mammals had achieved at least this second stage, that is, had both short- and middle-wavelength cone classes (Jacobs, 1981). The present investigation reveals a different arrangement in that the rat seems to have a single cone type with a peak sensitivity shifted only slightly to the long side of the rod. The rat has photopic vision but is a true monochromat. The rat may provide, therefore, a contemporary example of what might be envisioned as a first step toward the evolution of a color vision system like that characteristic of the primates.

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