Spectral Sensitivity, Photopigments, and Color Vision in the Guinea Pig (*Cavia porcellus*)

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Behavioral discrimination tests and electroretinogram (ERG) flicker photometry were used to measure spectral sensitivity and to define the spectral mechanisms of the guinea pig (*Cavia porcellus*). Results from these 2 approaches converge to indicate that guinea pig retinas contain rods with peak sensitivity of about 494 nm and 2 classes of cone having peak sensitivities of about 429 nm and 529 nm. The presence of 2 classes of cones suggests a retinal basis for a color vision capacity. Behavioral tests of color vision were conducted that verified this prediction: Guinea pigs have dichromatic color vision with a spectral neutral point centered at about 480 nm. The cone pigment complement of the guinea pig is different from that known to characterize other rodents.

*Cavia porcellus* has been so frequently used in scientific and medical investigations that its common name, guinea pig, has entered the English language as an informal descriptor for the subject of any sort of experimentation. In this role as subject, in addition to that as frequent household pet and occasional food source (Bradt, 1988), the guinea pig is surely among the most familiar of the domesticated rodents. Given that fact, it is remarkable how little is known about several basic features of the visual system and vision in the guinea pig. For instance, with regard to the identity of the photoreceptor and photopigment types and those visual behaviors most often associated with their variations (e.g., spectral sensitivity and color vision), there is sparse and often contradictory literature.

As for most other mammals, the guinea pig has a retina that is rich in rods. The rod photopigment is a typical mammalian rhodopsin with a peak absorption (\(\lambda_{\text{max}}\)) of about 497 nm (Bridges, 1959). There has been considerable inconsistency of opinion about the presence of cones in the guinea pig retina. Granit, who conducted many of the early studies of the electrophysiology of the guinea pig retina, routinely labeled the guinea pig as having a “pure rod-eye” (e.g., Granit, 1944, p. 103). Anatomists have offered variant opinions. O’Day (1947) reported that he was able to confirm the early observations of Kolmer (1936), who had detected cones in the guinea eye and concluded, “To the histologist, the retina of the guinea pig is certainly not “pure rod” (O’Day, 1947, p. 648). In the course of an analysis of the fine structure of the retina by electron microscopy, Sjostrand (reviewed, 1965) conducted a detailed examination of the synaptic terminals of guinea pig photoreceptors. He differentiated 2 types of photoreceptors in the guinea pig eye according to features of their synaptic morphologies. Some of these synaptic differences were of the sort that have in other cases been used to differentiate rods and cones (e.g., lateral extent of the synaptic surface), but Sjostrand argued these differences alone were not sufficient for determining whether a photoreceptor should be classed as a rod or a cone, and, thus, he too referred to the guinea pig as having a pure-rod retina (Sjostrand, 1965). There are apparently no other contemporary studies of the anatomy of guinea pig photoreceptors. In recent years, however, the presence of different cone types has been demonstrated through the use of various functional markers, including cone-specific, antivisual pigment antibodies. In particular, Szel and Rohlich and their colleagues have used two monoclonal antibodies that are visual-pigment specific to label “blue-sensitive” and “green and red-sensitive cones” in a number of mammalian species. In an article directed to another topic, Szel and Rohlich note that these 2 classes of antibody also label different populations of receptors in the guinea pig retina, thus providing evidence to suggest that the guinea pig retina not only contains cones but also may contain 2 different types of such receptors (Szel & Rohlich, 1992).

Electrophysiological studies have not clarified the picture. In recordings made from fibers of the optic nerve of the guinea pig, Granit (1947) could find no evidence for a Purkinje shift, a shift that he easily detected in parallel recordings made from cats. This result was in accord with his idea that the guinea pig has a pure-rod eye. However, he did find occasional elements in the optic nerve of the guinea pig that had very narrow spectral sensitivity curves. These elements, termed modulators by Granit, were recorded under conditions of photopic illumination, and their presence led to the speculation that the guinea pig had some photoreceptors that were functionally intermediate between typical rods and cones (Granit, 1947). In a later study, Dott and Wirth (1953) examined the behavior of a retinal gross potential, the electroretinogram (ERG), under conditions of intermittent light stimulation. Variation in stimulation rate and light intensity allowed a determination of the critical fusion frequency (CFF) of the ERG as a function of stimulus intensity. The curve so derived shows clear evidence for two limbs—one limb became asymptotic at stimulus frequencies at 10–25 flashes per second and a second limb showed a further increase in fusion frequency up to about 45–50 flashes per second. The appearance of such limbs in the CFF curve is classically taken as an indication of visual duplicity.

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(Hecht & Schlaer, 1936), and it accordingly suggests the presence of both rods and cones in the guinea pig. Thiele and Meissl (1987) have recorded light-evoked responses from single units in the pineal stalk and pineal body of the guinea pig. The average spectral sensitivity function from such units measured during light adaptation had a peak appropriate for rod input, at about 500 nm, but it also showed a curious secondary peak at about 450 nm. The latter could be interpreted as reflecting the contributions from receptors other than rods. That same conclusion may be drawn from an earlier study involving the measurement of lights reflected from guinea pig eyes (Weale, 1955). In that case, the appearance of separable bleaching spectra suggested the presence of multiple types of photopigment in the guinea pig eye. 

There are few behavioral studies of vision in guinea pigs. In seminatural and natural settings, guinea pigs and their caviid relatives show crepuscular activity rhythms (King, 1956; Rood, 1972); whereas in the laboratory, they may display essentially continuous activity under conditions of either constant illumination or constant darkness (Harper, 1976). These observations contain no strong inference about the receptor complement, but they do suggest that the guinea pig eye has a large dynamic intensity range. That range is typically associated with the joint presence of rods and cones. There appears to be only a single behavioral study of vision in the guinea pig that bears on the issues of concern here. Miles, Ratoosh, and Meyer (1956) sought to train an operant discrimination where reinforcement and nonreinforcement for lever-press responses were signaled by the presence of lights that varied in brightness, in color, or in both these dimensions. Although the guinea pigs rather quickly learned a brightness discrimination, they failed to show any discrimination between red and green light or between green and blue light. The authors concluded that guinea pigs lack color vision.

In light of the inconsistencies of results among these various studies, we have undertaken an electrophysiological examination of spectral mechanisms in the guinea pig retina and a parallel study of spectral sensitivity and color discrimination in the guinea pig. Our investigation was impelled by the lack of consistent information about basic aspects of vision in an important mammal and by the belief that there are now better methods for studying these issues than were available in the past, and also because recent studies involving phylogenetic analyses of amino acid sequence data have raised the intriguing possibility that the guinea pig may not be a rodent at all. Rather, it has been argued that the guinea pig may deserve, instead, a separate ordinal status (Graur, Hide, & Li, 1991; Li, Hide, Zharkikh, Ma, & Graur, 1992). If that idea is correct, then comparisons of the spectral mechanisms of the guinea pig with those of typical rodents could prove useful with respect to ideas about the evolution of receptors and photopigments.

Method

Subjects

Adult pigmented guinea pigs (Cavia porcellus) were obtained from a local supplier. Both males and females were examined. The guinea pigs were caged separately under standard colony conditions that included a 14:10-hr light–dark cycle. The guinea pigs that served as subjects in visual discrimination tasks were fed daily following testing in an amount sufficient to hold them at a constant body weight. The remaining guinea pigs were given ad-lib access to food.

Electrophysiological Experiments

Apparatus

The electrophysiological experiments involved an analysis of the flicker ERG in a version we have termed ERG flicker photometry. The basic features of the apparatus and the general procedures have been described in earlier publications (Jacobs & Neitz, 1987; Neitz & Jacobs, 1984). Only brief descriptions are given here.

The stimulus lights were produced with a three-channel, Maxwellian-view optical system. Lights from the three channels were optically combined to illuminate a 57° circular spot on the retina. The test light of the flicker photometer constituted one channel; it came from a tungsten halide lamp of a high intensity grating monochromator (Bausch & Lomb, Rochester, NY; half-energy passband = 10 nm). The other two channels also originated from tungsten halide lamps. One of these channels provided the test light of the photometer, and the other channel was used to produce an adaptation light. The intensity of the test light was varied by adjusting the position of a neutral-density wedge having an attenuation range of 3 log units. The intensities and spectral characteristics of the reference and adaptation lights were controlled by inserting neutral-density and color filters into the respective beams. All three lamps were underrun at 11 V from a regulated DC power supply, and each channel contained a high-speed electromagnetic shutter (Uniblitz, Vincent Associates, Rochester, NY). Light measurements were made with a silicon diode photodetector (PIN 10 DL, United Detector Technology, Culver City, CA).

Electrical signals were recorded with a bipolar, contact-lens electrode. A ground electrode was placed against the inside of the guinea pig's cheek. In the flicker photometry procedure, ERGs were elicited by temporally modulated, square-wave lights. Stimuli from the test and reference beams were interleaved such that there was a no-stimulus interval equal in duration to that of the test and reference lights between each successive light pulse. The frequency of stimulation was specified as the total number of light flashes per second. The ERGs elicited by test and reference lights were passed through a series of filter stages and electronically compared such that the signals from the two sources were cancelled when the two lights were equally effective at generating an ERG (Neitz & Jacobs, 1984).

Procedure

Guinea pigs were anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (33.3 mg/kg) and xylazine hydrochloride (6.7 mg/kg). The eye to be tested was dilated by topical application of atropine sulfate (0.04%) and phenylephrine hydrochloride. The guinea pig was positioned for recording in a modified stereotaxic instrument. Body temperature was supported with the use of a heating pad. Except for one experiment explicitly concerned with scotopic sensitivity, recordings were made in a room illuminated by overhead fluorescent lights yielding an illuminance of 296 lx.

To determine an ERG photometric equation, we averaged the responses to the lights for the last 50 cycles of a total presentation of 70 cycles. These equations were made by iteratively adjusting the neutral-density wedge in the test light beam until the test and reference lights were equally effective in producing an electric response. The dependent measure was the radiance of the test light required to achieve this equation. The wedge values at the point of equation were read to the nearest 0.01 log unit. All photometric equations were made at least twice during the recording sessions, and the separate values were subsequently averaged together. Stimulus flicker rate, test
wavelength, characteristics of the reference light, and adaptation state of the eye were varied. The particular combinations of these features are specified later in conjunction with the results from each experiment.

**Behavioral Experiments**

Detailed descriptions of the apparatus and procedure used to measure visual discriminations have been published (Jacobs 1983, 1984), so only a brief treatment is offered here.

**Apparatus**

The discrimination was set in the context of a three-alternative, forced choice. The guinea pig viewed three circular panels that were transilluminated from an optical system located outside the test chamber. There were two light sources; one was a tungsten halide lamp whose output was used to diffusely and equally illuminate each of the three panels (background lights). The other source was a grating monochromator (Model H-10, Instruments SA, Edison, NJ) having a 75-W xenon lamp. The output from this lamp was directed via a mirror system so as to illuminate any one of the three panels; it constituted the test light. Depending on the experiment, the test light was either added to the background or replaced it. Both lamps were run from regulated DC power supplies. Except for measurements of scotopic spectral sensitivity, the interior of the test chamber was always dimly illuminated to 100 cd/m².

The guinea pig was trained to discriminate the panel illuminated by the test light from the other two panels. To indicate its choice, the guinea pig touched a panel and, if the choice was correct, received a drop of liquid (Ocean Spray Cranapple Juice Cocktail). The location of the panel illuminated by the test light was varied randomly over trials, and the magnitude of the illumination difference between the positive and the two negative panels was titrated to determine discrimination thresholds. All aspects of the stimulus presentation, reinforcement delivery, and response monitoring were done automatically under program control of a computer.

**Procedure**

Using conventional procedures, 2 guinea pigs (1 male and 1 female) were first trained to select the uniquely illuminated panel when there was a large (> 2 log units) brightness difference between the test and background lights. After success at that task, they were tested in a series of experiments that fell into two categories.

Increment thresholds. Increment-threshold spectral sensitivity was measured under five different test conditions. In each case, monochromatic test lights (half-energy passband = 16 nm) were presented over a span of intensities in step values of 0.3 log unit. The intensity range was selected after initial training so that it yielded performance values running from about 80%–90% correct to 30%–40% correct. This typically required five or six intensity steps. Each of the intensity–wavelength combinations was presented in a three-trial block. The occurrence of the trial was signaled by a cuing tone; the trial terminated after the guinea pig responded or after 10 s without a response. A noncorrection procedure was used. Testing continued in daily sessions until a total of 100 or more trials had been accumulated at each wavelength–intensity combination. From these data, psychometric functions were drawn for each test wavelength by plotting mean percentage correct as a function of test light intensity. These points were fit to a logistic function having asymptotes of 100% and 33% correct with the variance and mean as free parameters. The function that provided the best least-squares fit to each data set was determined and from that the test stimulus intensity required to yield performance corresponding to the upper 99% confidence level was taken as the threshold value.

The several test conditions under which increment-threshold spectral sensitivity was measured were as follows: (a) Background—luminance = 0.92 log cd/m²; color temperature = 5350 K; test light—420–610 nm in steps of 20 nm. (b) Background—luminance = 1.73 log cd/m²; color temperature = 5350 K; test light—420–600 nm in steps of 10 nm. (c) Background—luminance = 1.73 log cd/m²; color temperature = 5350 K; test light—420–610 nm in steps of 10 nm. (c) Background—long wavelength light produced by the use of a high-pass filter having 50% transmission at 580 nm that yielded a luminance of 1.95 log cd/m², test light—420–580 nm in steps of 10 nm. A second set of spectral sensitivity functions was determined with these same conditions except that the luminance of the background light was increased to 2.91 log cd/m², (d) Background—short-wavelength light produced through the use of a 440 nm interference filter having a half-energy passband of 10 nm that yielded a luminance of either 1.3 cd/m² or 0.9 cd/m²; test light—550–630 nm in steps of 10 nm or 570–610 nm in steps of 5 nm. (e) Background—none and with the test light and the background lights had been calculated to be equally bright for the subject. The procedure used to establish these brightness levels is described later. The experiment was conducted for two different conditions of background lighting: (a) luminance = 2.43 log cd/m², color temperature = 5350 K; (b) luminance = 2.79 log cd/m², color temperature = 5350 K.

**Results and Discussion**

**Electrophysiological Measurements of Spectral Mechanisms**

To establish that ERG flicker photometry yields reliable data from guinea pig eyes and that the spectra measured with this technique can provide valid estimates of the photopigments, we first measured spectral sensitivity under scotopic test conditions for comparison with the earlier measurements of the rod photopigment in the guinea pig. Guinea pigs were adapted to the dark for 20 min and then tested in a fully darkened room. A slow flicker rate (8 Hz) and a dim achromatic reference light (radiance of 0.48 μW/cm² at the cornea) were used to enhance contributions from scotopic mechanisms. ERG flicker-photometric equations were obtained from each of 3 guinea pigs for test wavelengths taken at 10 nm steps from 430 nm to 590 nm. The resulting spectral sensitivity functions are shown in Figure 1. The solid circles represent log quantal sensitivity values at the level of the cornea, and the results for the 3 guinea pigs are arbitrarily spaced on the sensitivity axis. The sensitivity values for these subjects were greatly similar in that the range of sensitivity values for the 3 subjects at any of the test wavelengths did not exceed 0.1 log unit.
Figure 1. Scotopic spectral sensitivity functions for guinea pigs. Each individual symbol shows sensitivity as determined by electroretinogram flicker photometry from a dark-adapted eye. Results are shown for 3 guinea pigs; the data for each guinea pig are arbitrarily positioned on the ordinate. The continuous lines represent visual-pigment absorption curves determined as described in the text. The \( \lambda_{\text{max}} \) values and the goodness of fit (the least mean difference squared between the theoretical absorption curve and the data points) are, from top to bottom: 495 nm—1.61 \( \times 10^{-3} \) log unit; 492 nm—4.62 \( \times 10^{-4} \) log unit; 495 nm—9.38 \( \times 10^{-4} \) log unit.

To compare these scotopic spectral sensitivity functions with earlier estimates of the rod photopigment, we best fit each function with a photopigment absorption curve (continuous line in Figure 1). The procedure to accomplish the best fit was to shift a standard retinal \( \lambda_{\text{max}} \) photopigment absorption curve (Ebrey & Honig, 1977) along a log wavenumber axis (Baylor, Nunn, & Schnapf; 1987) in steps of 1 nm until the best least-squares fit was obtained between the data array and the pigment absorption curve. As can be seen in Figure 1, these fits are quite good for each of the 3 guinea pigs. The best-fitting curves for the scotopic functions of the 3 guinea pigs had \( \lambda_{\text{max}} \) values of 495 nm, 492 nm, and 495 nm. The average peak value (494 nm) is in reasonable agreement with the estimate for peak absorption of guinea pig rhodopsin (497 nm) reported by Bridges (1959). This correspondence suggests that ERG flicker photometry can provide sensitive and valid estimates of photopigments of the guinea pig.

Light adaptation and rapid flicker rates are among the tools traditionally used to diminish rod contributions to the ERG (Armington, 1974; Birch, 1989). We used both of these in an attempt to enhance any photopic components in the guinea pig ERG. As the results of Dodt and Wirth (1953) first suggested, such components are easily seen in the guinea pig ERG. Figure 2 shows spectral sensitivity functions obtained from 2 guinea pigs under conditions of light adaptation when the stimulus rate was increased to 40 Hz, and the reference light had an intensity of 74 \( \mu \) W/cm\(^2\). Relative to the scotopic spectral sensitivity functions, peak sensitivity was shifted to longer wavelengths (520–550 nm), and there was often a region of secondary elevation in the short wavelengths. Functions obtained under these test conditions are not accounted for by contributions from any single photopigment. Rather, the shape of the functions suggests that multiple spectral mechanisms (perhaps both rods and one or more classes of cone) underlie measured spectral sensitivity.

The spectral sensitivity functions of Figure 2 imply the presence in the guinea pig eye of at least one spectral mechanism that has a peak that is displaced toward the long wavelengths relative to that of the rod peak. We used three strategies to obtain an estimate of the spectral sensitivity of this mechanism. Each involved the use of a stimulus arrangement that would be expected to enhance contributions from this putative middle-wavelength sensitive (MWS) mechanism and to minimize contributions from other mechanisms having higher sensitivity to shorter wavelengths. These strategies involved using higher flicker rates or long-wavelength reference lights, or combining these features. In addition, we minimized the effects of contribution from short-wavelength mechanisms by restricting measurement to longer test wavelengths. The results from these three experiments are summarized as the spectral sensitivity functions of Figure 3. A total of 9 guinea pigs were tested under these three conditions. The left panel in Figure 3 shows spectral sensitivity functions obtained using more rapid flicker (62.5 Hz); the reference light was achromatic (intensity of 74 \( \mu \) W/cm\(^2\)). The middle panel shows functions obtained from 4 guinea pigs for experiments involving a 50-Hz stimulus and 590-nm reference light (50 \( \mu \) W/cm\(^2\)); the 2 guinea pigs with data shown in the right panel were tested using 40-Hz flicker in conjunction with a long-wavelength reference light (produced by inserting a high-pass filter having a 50% half-energy cutoff at 580 nm; intensity measured over the spectral range of 550–650 nm = 3.2 \( \times 10^{-5} \) W/cm\(^2\)).

Under the assumption that the spectral sensitivity recorded under these conditions might principally reflect contributions...
Figure 3. Spectral sensitivity measurements of the middle-wavelength sensitive mechanism of the guinea pig made with electroretinogram flicker photometry. Each data set shows the results obtained from 1 guinea pig. Results for individual guinea pigs have been arbitrarily positioned on the ordinate. Three different combinations of stimulus rates and reference lights were used: left panel (62.5 Hz, achromatic reference light); center panel (50 Hz, long-wavelength reference light); right panel (40 Hz, long-wavelength reference light). Each data set has been best fit with a visual-pigment absorption curve. The $\lambda_{\text{max}}$ value of each curve is indicated.

from a single photopigment, each of the spectral sensitivity functions was best fit with a photopigment absorption curve using the procedures described previously. These best-fit curves are shown in Figure 3 with the appropriate $\lambda_{\text{max}}$ values indicated. Over the restricted spectral range that was examined, these photopigment curves reasonably fit the data arrays. The spectral peaks varied modestly for the 9 subjects tested with three different procedures (mean $\lambda_{\text{max}} = 527.5$ nm; $SD = 2.3$ nm). Any residual contribution from shorter spectral mechanisms would have the net effect of causing the computed peaks of the best-fit functions to be shifted toward shorter wavelengths. It is thus reasonable to assume that these experiments indicate that the guinea pig retina contains a type of cone whose spectral peak is not shorter than about 528 nm. Given the good fit between the pigment absorption functions and the long wavelength slope of these spectral sensitivity functions, the actual peak is probably also not much longer than that value.

The spectral sensitivity functions of Figure 2 also suggest the presence in the guinea pig eye of a spectral mechanism having peak sensitivity that is shorter than that of the rods. A series of experiments were conducted to see if a more persuasive indication of such a mechanism might be obtained. The principal tools used to accomplish this were concurrent long-wavelength adaptation and short-wavelength reference lights. Figure 4 shows the average spectral sensitivity function obtained from 3 guinea pigs for 25-Hz flicker when the eye was concurrently adapted to an orange adaptation light (produced as in the right panel of Figure 3; radiance = $1.6 \times 10^{-5}$ W/cm$^2$). Under such conditions of adaptation, there is a relative enhancement of sensitivity to the short test wavelengths (compare Figure 4 and Figure 2). The differential distortion of the shape of the spectral sensitivity function with

Figure 4. Electroretinogram spectral sensitivity for guinea pigs obtained with 25-Hz flicker and concurrent long-wavelength adaptation. The solid symbols represent mean values obtained from 3 guinea pigs that were comparably tested. The vertical bar to the lower right shows the average total range of sensitivity for the 3 subjects.
chromatic adaptation indicates that multiple spectral mechanisms exist in the guinea pig retina. More importantly, these measurements suggest that there is indeed a short-wavelength sensitive (SWS) mechanism.

For 4 additional guinea pigs, we recorded spectral sensitivity functions at 25 Hz under yet more stringent conditions of adaptation (reference light of 440 nm at an intensity of 50 μW/cm² with concurrent orange light adaptation at a radiance of 3.2 × 10⁻³ W/cm²). The spectral sensitivity functions are given in Figure 5. Under these conditions, sensitivity to test lights of greater than about 500 nm was reduced so as to make these points unmeasurable. What remained were spectral sensitivity functions that show peak sensitivity in the vicinity of 420–430 nm. The functions for the 3 guinea pigs are very similar. The derivation and meaning of the fitted curve is described below.

In summary, measurements made with ERG flicker photometry suggest the presence of three spectral mechanisms in the guinea pig retina. One is a scotopic mechanism having a peak of about 494 nm. In addition, there are (at least) two spectral mechanisms operative under photopic test conditions. It proved comparatively difficult to completely isolate these mechanisms, but utilization of a range of different adaptation conditions suggests that one mechanism has peak sensitivity of about 420–430 nm and the other has peak sensitivity in the vicinity of 530 nm.

Behavioral Measurements of Spectral Sensitivity and Color Vision

Guinea pigs proved to be apt subjects for these tests of visual discrimination. Once trained, 2 guinea pigs produced highly reliable data throughout the 15-month period that was required to complete these tests. Results from our first attempt to measure a photopic spectral sensitivity function are shown in Figure 6. The solid symbols in Figure 6 represent the mean threshold values obtained from the 2 subjects. There are two notable features of these spectral sensitivity functions: (a) They have two locations of peak sensitivity, one in the short wavelengths in the vicinity of 420 nm and one in the middle wavelengths at 520–540 nm; (b) there is a dramatic decrease in sensitivity at a location intermediate to these peaks centered at about 480 nm.

The spectral sensitivity function of Figure 6 has several features characteristic of increment-threshold measurements obtained under photopic test conditions from many other mammalian subjects (Jacobs, 1993). In particular, the shape of the function suggests the presence of inhibitory interaction between two spectral mechanisms. Such inhibitory interaction can frequently be enhanced by increasing the intensity of the achromatic background light (Kalloniatis & Harwerth, 1990; King-Smith & Carden, 1976; Sperling & Harwerth, 1971). That was the purpose of the second experiment in which the
background light was substantially increased (by ca. 0.8 log unit) in intensity. These spectral sensitivity functions are plotted separately for the 2 subjects in Figure 7. As predicted, these functions show even more clearly the contributions to behavioral discrimination from two spectral mechanisms. Sampled at interval steps of 10 nm, the locations of peak sensitivity for the 2 subjects were similar—430 nm and 440 nm for the short mechanism and 530 nm for the other mechanism. The intermediate location of lowered sensitivity is even more apparent for this higher level of light adaptation and is again centered at about 480 nm. Although the shapes of the curves were similar for the 2 subjects, the curves differed in the relative heights of the two spectral peaks with one having higher relative sensitivity to the short wavelengths.

Full spectral sensitivity functions were measured for 1 guinea pig when the achromatic background light was replaced with an orange light. The intent of this experiment was to determine whether the two spectral mechanisms contributing to increment-threshold detection responded differentially to chromatic adaptation and to try to get a better estimate of the spectral properties of the SWS mechanism. The two spectral sensitivity functions that were obtained are shown in Figure 8. Note that the long-wavelength adaptation light caused a much greater loss of sensitivity to the long wavelengths than it did to the short wavelengths (compare Figure 7 and Figure 8). Increasing the intensity of this background light (bottom function of Figure 8) had a further differential adaptation effect in that the additional loss of sensitivity in the long wavelengths was much greater than it was in the short test wavelengths. The more intense orange light caused an additional threshold elevation of about 0.8 log unit at 560 nm, whereas the same light elevated threshold for a 430 nm light by only an additional 0.2 log unit. The experiment makes clear that the two components of the increment-threshold spectral sensitivity function of the guinea pig may be differentially adapted. We return below to the question of the spectral specification of the two underlying mechanisms.

Two additional experiments were conducted to better estimate the spectral properties of the MWS mechanism. In essence, the idea was to carry out behavioral measurements analogous to the electrophysiological measurements that were summarized in Figure 3. Spectral sensitivity measurements were made when the background light was of short wavelength (440 nm). Our intent was to diminish as much as possible any contribution from the SWS mechanism or the rods. Data were obtained at two different intensity levels; the test wavelengths were chosen to fall on the long wavelength slope of the MWS component. Results from the dimmer of these wavelengths are shown in the left panel of Figure 9; the results on the right were obtained after the background light had been slightly increased in intensity. In each case, the sensitivity data have been best fit by additive combinations of two photopigment absorption curves; these were 529 nm (18%) and 427 nm (82%) for the top data set, and 529 nm (11%) and 425 nm (89%) for the bottom data set.

A final spectral sensitivity measurement was made with background and test-chamber light extinguished. The single subject was adapted to the dark for a 20-min period before the initiation of each test session. The spectral sensitivity function obtained is shown in Figure 10. The threshold measurements

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Figure 8. Increment-threshold spectral sensitivity functions obtained from 1 guinea pig under two conditions of chromatic adaptation. In each case, the background was an orange light. For the results shown at the bottom, the background light was about 0.3 log unit more intense than the background used for the data shown at the top. The functions were best fit by additive combinations of two photopigment absorption curves; these were 529 nm (18%) and 427 nm (82%) for the top data set, and 529 nm (11%) and 425 nm (89%) for the bottom data set.

Figure 7. Increment-threshold spectral sensitivity functions for 2 guinea pigs. The data were obtained in the presence of an achromatic background light (1.73 log cd/m²). The results for the 2 guinea pigs are arbitrarily positioned on the ordinate. The curves are best-fit, subtractive combinations of two photopigment absorption curves. The values and relative proportions of these best-fit curves for the guinea pig at the top are 529 nm (21%) and 425 nm (79%); comparable values for the guinea pig at the bottom are 529 nm (45%) and 439 nm (55%).
Figure 9. Attempts to define the spectral positioning of the middle-wavelength sensitive (MWS) photopigment of the guinea pig from increment-threshold spectral sensitivity functions. Both panels show sensitivity measurements obtained with stimulus conditions intended to advantage contributions from the MWS mechanism. The background light in each case was 440 nm; the intensity of this light was higher for the data presented in the right panel (see text). The absorption curves for visual pigments drawn through the data sets was obtained by a best-fit procedure. The $\lambda_{\text{max}}$ values for the curves are 525 nm (left) and 529 nm (right).

Figure 10. Scotopic spectral sensitivity function obtained in a behavioral discrimination test from a dark-adapted guinea pig. The curve represents the visual pigment absorption function obtained by a best-fit procedure. The $\lambda_{\text{max}}$ of this curve is 498 nm (fitting error $= 8.86 \times 10^{-3}$ log unit).
of the test light to consistent avoidance. The intensity of the test light leading to this reversal was taken as indicating the location of the brightness match. Such matches were made for test wavelengths drawn from each end of the spectral range. Once these brightness matches were determined, the subject was trained in the color discrimination.

The left panel of Figure 11 shows asymptotic discrimination performance obtained across the spectral range tested for each subject. The plotted points are average discrimination recorded over the last 50 test trials for those test light intensities at the point of computed equal brightness. These functions are not materially changed either by averaging the results obtained at the equation value and for values ±0.1 log unit from this value or by simply plotting the poorest performance recorded at each test wavelength. These facts suggest that the discrimination results do reflect those from a test where there were no consistent brightness cues. The 2 guinea pigs performed similarly in this test. Both guinea pigs discriminated the spectral lights almost perfectly at either end of the test range; both guinea pigs also showed a loss of consistent discrimination in the vicinity of 480 nm. The horizontal dashed line of Figure 11 indicates the 95% confidence level. As judged by this performance criterion, the spectral range over which the guinea pigs failed to make a color discrimination extended from 470 nm to 484 nm for 1 subject, and from 477 nm to 482 nm for the other subject. The midpoints of this range are similar for the 2 subjects—477 nm and 479.5 nm, respectively.

The entire color discrimination experiment was run a second time after the achromatic lights had been increased in intensity by about 0.3 log unit. These results are shown in the right panel of Figure 11. The conventions were the same, and so were the results. In this case, discrimination failed for 1 subject for a spectral range of 471-482 nm (midpoint 476.5 nm) and failed for the other subject in a narrower range—from 477 nm to 483 nm (midpoint of 480 nm). These regions of discrimination failure define the presence of a spectral neutral point and verify the prediction that guinea pigs have dichromatic color vision.

General Discussion

Guinea Pig Photopigments and Spectral Mechanisms

The results reported here suggest that the eye of the guinea pig contains three classes of photopigment—one is a rod pigment and the other two are cone photopigments. Both electrophysiological and behavioral measurements allow estimates of the absorption spectra for these pigments. We noted that the spectral positioning of the MWS photopigment can be most reliably characterized by fits of absorption spectra to the long-wavelength portion of the curve. The ERG measurements (see Figure 3) and behavioral measurements (see Figure 9) are in good agreement in this regard. The former yielded an average $\lambda_{\text{max}}$ value of about 528 nm, whereas the behavioral measurement, which was most likely to have indexed activity of the MWS pigment alone (see Figure 9, right curve), gave a corresponding value of 529 nm. Because any residual contributions from other pigments having shorter $\lambda_{\text{max}}$ values would tend to shift the peak toward the short wavelengths, the longer of these two wavelengths is assumed as the best current estimate of the spectral positioning of the guinea pig MWS cone pigment.

By virtue of the considerable spectral overlap of the MWS and SWS photopigments in the short wavelengths, the spectral
positioning of the SWS photopigment may be best estimated from those experiments in which contributions from MWS receptors were reduced through the use of chromatic adaptation. These are the ERG measurements shown in Figure 5 and the behavioral measurements in Figure 8. In these experiments, even with concurrent long-wavelength adaptation, there likely remains some residual contributions from the MWS pigment. Accordingly, to estimate the spectral positioning of the SWS pigment we assumed that the ERG and behavioral results of Figures 5 and 8 represent summative contributions from MWS and SWS mechanisms. To determine the spectral positioning of the SWS mechanism, the array of sensitivity values were best fit using summative combinations of a photopigment having $\lambda_{\text{max}}$ value of 529 nm and an SWS photopigment. The spectral position of the putative SWS photopigment was varied in steps of 1 nm (over the spectral range of 420-440 nm) and in relative proportions of 1% until the best-fitting combination of the two pigments was obtained. These best-fit curves are the continuous lines drawn through the data in Figures 5 and 8. This strategy accounts quite well for the shape of the spectral sensitivity functions. The estimated peak values for the SWS mechanism show relatively small variability. The 4 subjects whose ERG results are shown in Figure 5 have an average SWS $\lambda_{\text{max}}$ value of 427.3 nm ($SD = 3.1$); the subject of the behavioral experiments shown in Figure 8 yielded corresponding values of 425 nm and 427 nm for the two experiments.

The increment-threshold measurements of Figures 6 and 7 also show clear evidence of inputs from both cone types. The combination of these inputs in this situation, as for other increment-threshold measurements on achronic backgrounds (e.g., Kalloniatis & Harwerth, 1990; Sperling & Harwerth, 1971), involves inhibitory interaction between the two mechanisms. These data were best-fitted by using a strategy earlier used to account for such functions obtained from other dichromatic subjects (Jacobs, 1990; Neitz, Geist, & Jacobs, 1989), that is, by searching for the best subtractive combination of a 529-nm MWS pigment and an SWS photopigment. The best-fit functions so obtained are given by the curves of Figures 6 and 7. This strategy provides a reasonable account of the discrimination data. Taken together, the summative and subtractive fits to the electrophysiological and behavioral data yielded a total of nine individual estimates of the spectral positioning of the SWS cone pigment of the guinea pig. The range of such estimates was 13 nm; the mean estimate was 429 nm.

The behavioral and electrophysiological measurements of scotopic spectral sensitivity (see Figures 1 and 10) are in reasonable agreement with each other and with the earlier spectrophotometric measurements of rod pigment in the guinea pig (Bridges, 1959). All these measurements suggest that the rods of the guinea pig contain a pigment whose peak sensitivity is in the range from about 494 nm to 498 nm.

Two other issues of concern in deriving estimates of photopigment spectra from spectral sensitivity measurements deserve mention. One is that in producing these estimates no corrections have been made for possible preretinal filtering of the spectral input. The principal source of such filtering in the eyes of mammals is lens pigmentation (Muntz, 1972). Cooper and Robson (1969) measured density spectra for the intact lens of the guinea pig, and their measurements show little spectrally selective light losses in the guinea pig lens over the portion of the spectrum examined in the present experiments. Accordingly, we concluded that no corrections need be made for preretinal filtering. A second source of general concern in comparing pigment absorption spectra and spectral sensitivity measurements are possible effects of pigment self-screening. It has long been recognized that the shape of the absorption spectrum of a photopigment depends on pigment concentration (Dartnall, 1957) and that comparisons of action spectra (such as the spectral sensitivity curves of the present experiment) with standard photopigment absorption curves may be inaccurate if there is substantial pigment density. There are no direct measurements, nor indeed any other information, to allow an estimate of the optical density of guinea pig photoreceptors, but several aspects of the results suggest that not much improvement on the spectral estimates could be obtained by consideration of optical density. In the case of cones, this may be true because all of the ERG measurements and most of the behavioral measurements were obtained from highly light-adapted eyes. This would tend to greatly reduce pigment density and a priori would seem to obviate the need for concern about the effects of this variable. On the other hand, one might expect that the scotopic measurements would reflect the operation of rods whose pigment density was near maximal. The fact that the photopigment absorption curves (see Figures 1 and 10) provide a good account of the shape of the spectral sensitivity curves suggests that any effects of self-screening must be small. Why this should be so is unclear, but it may be noteworthy that similar uncertainty surrounds comparisons of human rod vision and rhodopsin (Alpern, Fulton, & Baker, 1987; Bowmaker & Dartnall, 1980).

We noted previously that the earlier studies of the spectral mechanisms of the guinea pig were inconsistent in outcome. However, one can perhaps find in them previews of the present results. For instance, the narrow-band “modulator” elements detected in early studies of the discharges of optic-nerve fibers by Granit (1947) have peaks of about 450 nm and 530 nm. Spectral peaks of about 460 nm and 530 nm also appear in bleaching spectra obtained from retinal densitometric measurements (Weale, 1955). The spectra determined from visually evoked responses of the guinea pig pineal gland show a secondary peak at about 450 nm (Thiele & Meissl, 1987). In each of these cases, the reported spectral peak values are close enough to the current measurements of the guinea pig cone pigments to suggest these earlier results might have reflected signals of cone origin in the guinea pig visual system.

**Color Vision**

Given the historical arguments about whether the retina of the guinea pig contained any cones at all and the emphatic denial of color vision in the guinea pig in the only study directed to that end (Miles et al., 1956), it was somewhat surprising to find that cone signals can be easily detected both electrophysiologically and behaviorally and that color vision can be readily demonstrated. Like many other mammals, the guinea pig is a dichromat. That diagnosis rests on the fact that...
both subjects were able to discriminate monochromatic lights from equally bright achromatic lights except when the mono-
chromatic light was set to a narrow wavelength range (see Figure 11). The wavelength of the monochromatic light that matched in appearance an achromatic light for the guinea pig was located in the vicinity of 480 nm. This location, the neutral point, represents a color match the details of which depend on the spectral positioning of the two cone pigments, as well as the effective spectral characteristics of the achromatic light. For dichromatic observers, the neutral point effectively splits the spectrum such that subjects can make color discriminations between lights whose spectral energy distributions fall principally to either side of the neutral point. Dichromatic observers also can discriminate most stimuli having narrow-band spectral energy from stimuli having a continuous spectrum (see Figure 11). In the previous experiment on guinea pig color vision, the discriminations tested were between a red and a green light and between the same green light and a blue light (Miles et al., 1956). These stimuli were produced by passing light through broad-band filters. It seems unlikely that the first discrimination would have been possible for the dichromatic guinea pig, because the effective energies of both lights were probably restricted to the same side of the neutral point. On other hand, one might expect that the guinea pigs would have been able to discriminate a blue light from a green light. We can only speculate why they did not; but whatever the reason, the conclusion of the earlier study seems to be in error. Guinea pigs do have color vision. Furthermore, the ease of demonstrating color vision in a laboratory test suggests that it may provide a useful additional source of information about the environment of the guinea pig.

Phylogeny of Photopigments

There is only limited information about the distribution of cone photopigments among the mammals. Even so, it may be useful to briefly consider the cone pigment complement of the guinea pig from a comparative perspective. What has so far been learned about the cone pigments of rodents has been recently summarized (Jacobs, 1993). In brief, several different cone pigment arrangements have been documented for rodents. Species from the family Sciuridae (the squirrels) typically have two classes of cone pigment. All squirrels (with the possible exception of the nocturnal flying squirrel) have an SWS cone pigment with a peak somewhere in the range from about 435 nm to 445 nm. In addition, there is a class of MWS cone whose peak location varies—among the ground-dwelling squirrels this pigment has a peak of about 520 nm, whereas for the arboreal squirrels the corresponding peak is about 543 nm. Cone pigments have also been measured for a number of species from the family Muridae (mice, rats, etc.). Many of these species also have two classes of cone photopigment. However, one of these pigments has a very short wavelength value—in the ultraviolet at about 360 nm. The other (MWS) pigment varies among species with peak values that extend from about 493 nm to about 512 nm. In addition, there is evidence to suggest that some murid rodents may have only a single cone pigment with peak in the vicinity of about 500 nm. Beyond these two large families, few other rodents have been exam-


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