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(\lambda_{\text{max}} = 500) participated in the light reflex. © 1982.

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RETINAL SENSITIVITY MEASURED BY THE PUPILLARY LIGHT REFLEX IN RCS AND ALBINO RATS

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Abstract—The effects of retinal degeneration on the sensitivity of the retina were studied in the Royal College of Surgeons (RCS) rat by measuring the light reflex of the pupil in response to ganzfeld (full field) flashes. Light reflex thresholds were measured for animals from 32 to 683 days of age, and an age-related decrease in sensitivity of 5.2 log units (maximum) was measured. In contrast, thresholds for non-dystrophic albino controls increased only slightly during a comparable period. RCS rat thresholds increased more for short wavelength light than for long wavelength light. The end result was an altered action spectrum of the light reflex which largely, but not exclusively, reflected cone function. Even in cases of advanced degeneration the light reflex thresholds we measured showed significant input from rods. Pupillary dark adaptation measured following ganzfeld bleaches (10%) with test stimuli of two different wavelengths revealed two mechanisms, a photopic mechanism ($\lambda_{\text{max}} = 520$) determined thresholds early in dark adaptation, but later a scotopic mechanism ($\lambda_{\text{max}} = 500$) participated in the light reflex.

INTRODUCTION

The Royal College of Surgeons (RCS) rat suffers from hereditary retinal degeneration (Dowling and Sidman, 1962) and has served as an animal model of the class of inherited human diseases, retinitis pigmentosa (Young, 1977). Retinitis pigmentosa (RP) is a retinal degeneration syndrome with various modes of inheritance. The most commonly reported initial symptom is night blindness which may initially spare the central region. However, photopic vision and central vision eventually fail in most cases where the patient reaches a sufficiently advanced age (for a review see Krill, 1972).

In comparison with what is known about the etiology of retinitis pigmentosa there exists a relative wealth of data concerning the nature and the time course of the physical changes which occur in the retina of the RCS rat (e.g. Bourne et al., 1938; Cicerone et al., 1979; Dowling and Sidman, 1962; LaVail et al., 1974; Lucas et al., 1955, Noell, 1977). From the work of Cicerone et al. and of LaVail et al., it is known that both rods and cones degenerate, but the rods do so more extensively, and in a shorter period of time. The result is a drastic change from mostly rods in the retinas of young RCS rats to mostly cones in rats with advanced degeneration. Accordingly, Cicerone et al. have shown that all ganglion cells with measurable sensitivity in RCS rats with advanced degeneration appear to be driven by cone inputs. On the other hand, the spectral sensitivity of single ganglion cells in normal rats invariably matches a rhodopsin action spectrum when measured under the same (dark adapted) conditions. Although the exclusive presence of units with photopic sensitivity in the RCS retina provides evidence for the selective survival of cones, it is conceivable that many scotopic units remain undetected. In fact, ganglion cells with low sensitivity are routinely encountered in the optic tract of older RCS rats (Cicerone et al., 1979).

In mammals, the pupil responds to a flash of light with a transient constriction known as the pupillary light reflex. The relative ease of observing the light reflex and its high sensitivity motivated our use of this response to estimate retinal sensitivity. In humans, the evidence that the receptors which mediate vision are the same receptors responsible for the light reflex is compelling (Lowenstein and Loewenfeld, 1969). The direct light reflex of the rat is similar to that of humans, but unlike humans, the existence of a consensual pupillary light reflex in rats has not been demonstrated (Lowenstein and Loewenfeld, 1969). As a control for possible extraretinal influences we determined at the outset of this study that cutting the optic nerve of the rat's eye abolished the pupillary light reflex. This confirmed that in the rat, as in humans, the light reflex depends on stimulation of photoreceptors in the retina. The pupillary system has a considerable gain and a unitary response, presumed to reflect activity integrated over large retinal areas (Bouma, 1965; Schweitzer, 1955). These characteristics made the pupillary light reflex an ideal response with which to study the absolute sensitivity of the retina to light and the spectral sensitivity throughout the lifetime of the RCS rat. The pupillary light reflex measurements we report here provide evidence for a rod contribution persisting in advanced degeneration. This input, although reduced by over 4 log units as
compared to the normal, is reflected in the pupillary light reflex of animals as old as 297 days postnatal. A preliminary report of some of these findings has been presented elsewhere (Trejo and Cicerone, 1978).

METHODS

Animals

Eleven pink-eyed beige and tan hooded RCS rats were selected as subjects from a colony maintained at the University of California, San Diego. Our colony was derived by mating of siblings from RCS rats descended from the colony originally maintained at Harvard University. Six Sprague-Dawley albino rats served as controls.

All rats were reared from birth in a darkened room where light exposure was limited to brief periods of illumination from red overhead lights during cage cleaning and during preparation of the animals for experiments. Ophthalmoscopic examinations revealed no cataracts or other gross ocular anomalies in any of the animals used in this study.

Apparatus

A uniform, full-field illumination of variable wavelength and intensity was presented to the eye of the rat (see Fig. 1). The pupil of the eye was viewed continuously with the aid of an infrared sensitive television camera (Panasonic WV-1350) and a video monitor. Responses of the pupil to ganzfeld flashes were also videotaped for later, more detailed analyses.

Procedure

Animal preparation. Each animal was dark adapted for a minimum of 12 hours before each experimental session. Under dim red illumination and mid ether narcosis the animal was placed in a restraining jacket and a restraining box. The box was placed on a platform with the animal's head protruding into the integrating sphere and 15-30 min was allowed to ensure complete recovery from the ether exposure.

Threshold measurements. The threshold criterion was a transient, 0.2 mm constriction in the diameter of the pupil in response to a 500 msec flash. The observer waited until the pupil diameter was within about 1 mm of its dark adapted diameter and the animal was still before delivering a test flash. Since the latency of the light reflex was stable, the observer could discount other pupillary movements which were randomly related to stimulus onset.

Thresholds were determined for calibrated combinations of interference filters (Ditric Optics, Marlboro, MA, 3 cavity, 8-12 nm bandwidth at half height) and neutral density filters (Oriel) in the beam. The sequence of wavelengths was quasirandomized with the restriction that the 500 nm filter was always used at the beginning, in the middle, and at the end as a check for changes in the level of adaptation during the session.

Time-course and intensity-response functions. The amplitude of pupillary constriction was measured as a function of the intensity of the stimulus to yield intensity-response functions. The luminance of the stimulus was systematically increased in 0.5 log unit steps, and the corresponding pupillary movements were videotaped. During analysis the sequences were viewed on a video monitor at an overall magnification of 19.7× and measurements were made on stopped images at 0.7 sec steps. These measurements were used to track the time course of the light reflex (see Fig. 2). The maximum pupillary constriction for each stimulus obtained from these measurements was used to plot the intensity-response functions. Our measurements showed that the slopes of these functions did not vary significantly with wavelength (e.g. Fig. 6).

Dark adaptation. Pupillary sensitivity to light of 500 and of 600 nm was measured during recovery from exposure to a white ganzfeld bleach produced by a 30 sec exposure to the full output of a 150 W xenon arc lamp in the integrating sphere. In this condition the sphere produced an irradiance of 12.36 log quanta mm² at the corneal surface. Using Cone's (1963) analysis of Dowling's (1963) 5 min bleaches we estimated that this light bleached about 10% of the pigment of the retina. (The time-weighted average pupil diameter during the bleach was 0.8 mm, and this value was used in the calculation.)
An experienced observer decided when a stimulus produced a threshold constriction. As a check on this method three entire dark adaptation sequences were videotaped and subsequently analyzed as described above. This more objective method produced dark adaptation curves which did not differ systematically from those produced by the observer's judgments. Due to the speed of recovery and to the necessity of changing filters manually the number of points obtained for each run was not large.

Calibration. The irradiance at the cornea with each interference or neutral density filter interposed in the light beam was measured with an electronic radiometer (Model 450, E. G. & G. Electro-optics Division; Salem, MA) positioned at the location occupied by the rat's eye.

Since we lacked comparative data concerning the relative size and light gathering properties of the eyes of RCS rats and of the control strain, all stimulus values are reported in units of corneal irradiance. To estimate the retinal irradiance produced by our threshold stimulus for the control animals, Cone's (1963) estimates of 12% absorption by the ocular media, 23% absorption of 500 nm light by rods, and $40 \times 10^4$ rods $\text{mm}^{-2}$, and Block's (1969) estimates of 2.97 mm for the posterior nodal distance and 1.34 for the index of refraction at the cornea of the albino rat eye were used. Our measured values of corneal irradiance of our 500 nm light ($5.38 \log$ quanta set$^{-1}$ mm$^{-1}$) and pupil diameter (3 mm) were used to compute a value of $1.75 \times 10^{-1}$ quantum absorbed rod$^{-1}$ set$^{-1}$ at threshold.

RESULTS

The pupillary light reflex

Plots of net pupillary constriction as a function of post-stimulus time at six levels of intensity over a four log unit range, for a 361-day old albino control appear in Fig. 2. At threshold stimulation (0.2 mm decrease in pupil diameter) the pupil begins to con-
light reflex for RCS animals older than 135 days of age exhibits two phases of constriction at high intensity levels. Furthermore, the older the RCS rat, the more light is required to attain a specified maximal constriction as compared to the 361-day old control. An indication of variability in these plots is given by the error bars for the 524-day old RCS rat. These indicate two standard errors of the mean (SEM) for the most variable data points of the set of plotted averages.

**Pupillary sensitivity vs age**

Pupillary ganzfeld thresholds for four albino control rats are plotted in Fig. 4. The corneal irradiance at threshold of our 500 nm light stimulus is plotted as a function of postnatal age in days. Measurements were made between 53 and 705 days of age with multiple measurements made on certain individuals.

In the same figure thresholds are plotted for 11 RCS rats ranging between 32 and 683 days of age. For the RCS rats, thresholds increase steadily between 32 and 683 days of age, resulting in progressively larger threshold elevation relative to controls. Exponential functions were fit to each set of data, and these are shown as solid lines in Fig. 4. The equation for the function describing the RCS rat data is given by

$$\log I(t) = 7.5 - 5.8 e^{-t/303}$$

and that for the albino control data by

$$\log I(t) = 3.5 - 1.9 e^{-t/500},$$

where $I$ is the threshold irradiance in quanta sec$^{-1}$ mm$^{-2}$ at the cornea and $t$ is age. The error bars shown at the right of each curve represent two standard deviations of the discrepancy between data and prediction for each model. The time constant of the fit to the RCS data is significant ($F_{1, 23} = 191.74$), predicting 4.62 log units of threshold elevation between 31 and 683 days. The time constant of the albino fit is also significant ($F_{1, 19} = 11.56$), predicting 1.25 log units of threshold elevation between 53 and 705 days.

**Intensity-response functions**

The maximum pupillary constriction for different intensities of a 60°, 500 nm flash of 500 msec duration is plotted in Fig. 5 for a control rat and for RCS rats of 31, 165 and 298 days of age. (In preliminary experiments, pupillary responses were measured in response to a 60° field flashed on a diffusing screen which the animal viewed from the restraining box.) The function for the 31-day old RCS resembles that of the control closely enough to permit superposition if it is displaced horizontally by 1.7 log units. implying that the same mechanisms mediate the light reflex in the normal and in the 1-month old RCS rat.

Superposition of the intensity-response functions of the 165- and 298-day old RCS rats upon that of the control is not possible. Thus it is apparent that in addition to overall losses of sensitivity there are changes in the relative sensitivity of the mechanisms which mediate the light reflex at different intensities.

![Fig. 4. Pupillary light reflex thresholds for albino control rats and for RCS rats of different ages. The ordinate gives the intensity of a 500 nm, ganzfeld flash (500 ms) in log quanta sec$^{-1}$ mm$^{-2}$ measured at the cornea. The abscissa indicates postnatal age in days. Thresholds of the four albino control rats were measured at 53, 78, 166, 203, 208, 281, 361, 427, 433 and 705 days of age. Different open symbols denote different control animals. The line through the control thresholds is described by an exponential function, $\log I(t) = 3.5 - 1.9 e^{-t/500}$, where $I$ is corneal irradiance and $t$ is age in days. Thresholds of 11 RCS rats at various ages are marked by closed symbols. Measurements for RCS rats were made at 27, 32, 45, 52, 81, 82, 84, 86, 92, 112, 132, 157, 244, 315, 389, 432, 494, 524, 541, 589, 663, 666 and 683 days of age. Different animals are indicated with different symbols. The function drawn through the RCS rat data has an equation of the form $\log I(t) = 7.5 - 5.8 e^{-t/303}$, where $I$ is corneal irradiance and $t$ is age in days.]
Intensity-response functions measured with a 500 and 600 nm test are plotted for the control rat and the 31- and 298-day old RCS rats in Fig. 6. Each set of points has been scotopically equated by shifting the set generated by the 600 nm light to the left 1.38 log units, the difference required for a Dartnall nomogram curve peaking at 500 nm (Ebrey and Honig, 1977). This results in a superposition of the lower branches of the curves for the two wavelengths and shows that the action spectrum of the pupillary light reflex at scotopic levels is likely to be that of rhodopsin. Even for the 298-day old RCS rat, at lower
Fig. 7. Spectral sensitivity of the pupillary light reflex for a 205-day old albino control rat measured in a ganzfeld. Thresholds at each wavelength were determined from observations of the light reflex on the video monitor. Each point corresponds to the relative intensity of the stimulus required to produce a 0.2 mm pupillary constriction. The ordinate gives the log relative sensitivity of the light reflex with the value for 500 nm light arbitrarily set equal to zero. The abscissa gives the wavelength of the stimulus. The dashed curve is a Dartnall nomogram curve with a peak near 500 nm.

At high intensities the action spectrum of the pupillary light reflex appears to be that of rhodopsin.

The separation in the upper branches of the curves corresponds to a gain of 0.7 log unit for the 600 nm stimulus at high intensities.

This difference is consistent with a nomogram curve for a pigment with a peak absorption near 520 nm, and thus the data suggest that at higher intensities the light reflex is driven by a mechanism whose action spectrum has a peak wavelength near 520 nm.

Spectral sensitivity of the pupillary light reflex

The dark adapted pupillary spectral sensitivity of a control rat measured in the ganzfeld at 205 days of age is plotted in Fig. 7. A Dartnall nomogram curve with a peak of 500 nm was eye-fit by vertical sliding. In no case was the pupillary spectral sensitivity of any of the control animals fit better by a nomogram curve of a significantly different peak wavelength.

In Fig. 8, the upper set of points is the mean spectral sensitivity derived from two separate sessions in the ganzfeld for a single RCS rat at 35 and 37 days of age. The points determined from threshold intercepts of intensity response functions for a 363-day old RCS rat. The dashed curve is a 500 nm Dartnall nomogram; the solid curve is a 520 nm Dartnall nomogram curve.

The same kind of dark adaptation experiments were conducted for a 157-day old RCS rat and a 297-day old RCS rat, the results of which are also consistent with rods determining threshold at these times. There is a clear and consistent separation of the 500 nm data points from those for 600 nm light, which is consistent with rods determining threshold at these times. There is a clear and consistent separation of the 500 nm data points from those for 600 nm for earlier times, suggesting that thresholds soon after the bleach are determined by a photopic mechanism.
shown in Fig. 9. As with the albino control, thresholds appear to be photopically determined early in dark adaptation, while later they appear to be scotopically determined. For these RCS animals, thresholds are generally higher than those for the control at comparable times after the bleach. Also, the change from photopic to scotopic determination of thresholds occurs later, near 40 min after the bleach.

We measured dark adaptation in three other RCS rats at ages of 76, 85, 122 and 297 days and in three other albino controls at ages of 66, 114, 196, 279 and 433 days. In every case we found clear evidence for scotopic as well as photopic function in dark adaptation. The RCS animals consistently showed higher thresholds and a change from photopic to scotopic determination of thresholds which occurred later than in the controls. Thus, all our data is consistent with the examples we show.

DISCUSSION

Pupillary sensitivity with age

The intensity-response functions in Figs 5 and 6 show that there is an overall loss of sensitivity and a change in the relative sensitivity of the low intensity and high intensity components of the light reflex of the pupil with age in the RCS rat. As the retina degenerates, progressively more light is required to produce a given constriction.

We find that the increase in RCS thresholds (Fig. 4) can be modeled as an exponential loss of sensitivity. The RCS thresholds rise steadily after about 30 days postnatal, where they approximate control thresholds. Using a value of corneal irradiance of 7.5 log quanta sec\(^{-1}\) mm\(^{-2}\) as the asymptotic value, \(k_1\), we fit the RCS thresholds of Fig. 4 to the model

\[
\log I(t) = k_1 - k_2 e^{-t/k_3}
\]
from which equations (1) and (2) were derived (see Fig. 4). The least squares estimates were \( \tau = 303.03 \) days and \( k_s = 5.83 \) log quanta sec\(^{-1}\) mm\(^{-2}\). This equation predicts a threshold of 2.22 log quanta sec\(^{-1}\) mm\(^{-2}\), at 30 days postnatal which agrees closely with the control thresholds. The agreement of the curve plotted from this equation and the data of Fig. 4 is quite good, but we cannot deny that this data would also be consistent with other models. We did attempt linear fits to the data, which accounted equally well for the variability of data. However, the nature of the linear fit was unsatisfactory, for the residuals were generally negative for high ages, positive for middle ages, and negative for early ages. The same cannot be said for the albino data where exponential and linear fits were equally satisfactory.

The electoretinogram also diminishes with age in RCS rats, but it is impossible to measure beyond about 90 days postnatal (Dowling and Sidman, 1962; Perlman, 1978). Our pupillary light reflex thresholds extend the range of retinal sensitivity measurements to about 700 days and suggest prolonged persistence of functional photoreceptor elements in the RCS retina.

The decline in sensitivity of the RCS rats was probably not significantly altered by repeated exposures to the testing situation. Close inspection of Fig. 4 reveals that the first tests performed for several animals were performed at various advanced ages (each animal in Fig. 4 is represented by a unique symbol). The thresholds in these first tests fit well within the overall pattern of age-related sensitivity loss. There is no consistent trend towards lower thresholds in these first exposures as we might expect if the testing situation had significantly accelerated the loss of sensitivity.

### Spectral sensitivity

The dark adapted spectral sensitivity of the pupillary light reflex measured in control rats is similar to that in the RCS rat at 1 month of age (Figs 7 and 8). At intermediate stages of the disease there is a relative increase in the effectiveness of long wavelength light (Fig. 8, 216-day old animal) and in later stages (Fig. 8, 363 day-old animal) pupillary sensitivity may be photopically driven. When a photopic action spectrum is obtained, it appears to be well fit by a Dartnall nomogram curve for a single visual pigment with peak absorption near 520 nm. In parallel with this finding, the peak of the pupillary spectral sensitivity curve is displaced toward longer wavelengths in RP patients with severe sensitivity loss (Alexandridis and Weddigen, 1971). In both cases the wavelength of peak sensitivity is displaced, presumably owing to the change in the composition of the pupillomotor receptor population.

The change in the spectral sensitivity of the light reflex (Figs 6 and 8) is probably the result of the changing proportion of rods to cones in the RCS retina (LaVail et al., 1974, Cicerone et al., 1979). Our measurements show that the pupillary light reflex exhibits input from a scotopic mechanism even in cases of advanced degeneration (298 days of age). To our knowledge this is the first study to show the functional involvement of a rhodopsin-based mechanism at such advanced age in the RCS rat. It is likely that the pathways which mediate the light reflex can integrate activity over large retinal areas (Schweitzer, 1955; Bouma, 1965). The gain introduced by pooling over such large areas could suffice to provide a scotopic input to the pupillary light reflex even with very few rod photoreceptors remaining.

### Dark adaptation

During dark adaptation in the control rats a change in the relative sensitivity of the pupillary system to 500 and 600 nm lights occurs in the functions defining threshold. This feature is consistent with photopic determination of thresholds early in dark adaptation and scotopic determination subsequently. Although it may be difficult to distinguish more than one branch in the dark adaptation curve of an RCS rat with advanced degeneration there is a change in the relative sensitivity to light of 500 and of 600 nm. Cicerone et al. (1979) found that the Dartnall nomogram curve which matched the spectral sensitivity of ganglion cells in older RCS rats had its peak at 520 nm. The relative sensitivity difference to lights of 500 and 600 nm predicted by the 520 nm nomogram is a factor of 0.79 log unit (Ebrey and Honig, 1977). This value is close to the relative sensitivity difference for 500 and 600 nm lights measured early in dark adaptation in both the RCS and albino rats we examined. Birch and Jacobs (1975) who used a behavioral method, and Cicerone (1976), who used the electoretinogram, have also reported a visual mechanism in the rat with peak sensitivity at 520 nm. This suggests that the same receptor pigment(s) which underlies ganglion cell response in advanced retinal degeneration also determines the photopic spectral sensitivity of the pupillary system of the rat.

In the late phase of dark adaptation in both RCS and control rats, the mean difference in sensitivity to 500 and 600 nm lights approximately matches the absorption spectrum of rat rhodopsin (Bridges, 1959) or a 520 nm Dartnall nomogram curve (Ebrey and Honig, 1977). Thus it appears that the scotopic inputs to the pupillary system in both groups of rats also depend on the same receptor pigment, rhodopsin.

The changes in the two functions describing dark adaptation and in the time to branching between these functions shown by the RCS rat data are consistent with a model of the disease in which receptor damage and subsequent sensitivity loss progress at different rates for rods and cones. In dark adaptation, the sensitivity of the mechanisms driven by rods or cones is inversely related to the vertical position of the functions which describe threshold in the dark adaptation plot. With age, both mechanisms "rise" in the plot but the scotopic mechanism rises faster. Eventually only the tail of the scotopic mechanism
may be detectable as the rest is hidden above the photopic one. As a result, the time at which the scotopic mechanism begins to determine thresholds will occur progressively later in dark adaptation. It should be noted here that the qualitative changes in the shape of the pupillary dark adaptation function which correlate with the severity of visual sensitivity loss in RP patients reported by Alexandridis and Weddigen (1971) and by Berson et al. (1969) are similar to the pattern of age related changes we see in this study for RCS rat dark adaptation.

**Time-course of response**

At suprathreshold levels of stimulation we noticed an anomaly in the time-course of pupillary constriction in some RCS rats over 5 months of age (Fig. 3). Specifically, constriction occurred in two distinct phases separated by an inflection. The time required for the pupil to redilate appeared to increase with age in RCS rats. Such responses have not been observed in unaffected controls and have not been described in any of the literature known to us. Near threshold, the responses of RCS rats and controls are similar, though the RCS rat responses appear to be slightly slower. Since we measured only the peak constriction, this should not affect any of the results which depend on threshold data. For the intensity-response functions, the first peak was chosen as the response value whenever multiple peaks occurred in the response. These time-course differences may prove important in characterizing the temporal properties of the visual system in RCS rats.

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