Cone Inputs to Ganglion Cells in Hereditary Retinal Degeneration

Abstract. *The photoreceptor layer degenerated, but cone nuclei apparently devoid of outer segments were retained in retinas of aged rats of the Royal College of Surgeons strain from which optic tract activity was recorded. Measures of sensitivity showed these single axons of retinal ganglion cells to have photopic spectral responses. Cone remnants containing a cone pigment may be the photoreceptive elements in these retinas.*

Retinal cellular degeneration as a consequence of inherited disease occurs in many species (*1*). The process of degeneration may reveal aspects of cellular structure, function, or interactions that could characterize the disease or yield information about healthy cell life.

The progressive degeneration of the retina in the Royal College of Surgeons (RCS) strain of rats results in a steady decline in the electroretinogram (ERG) and morphological changes beginning with an accumulation of extracellular lamellae between the photoreceptors and pigment epithelium (*2, 3*). According to early reports, the retina is ultimately denuded of photoreceptor cells, but the rest of the retina remains, by comparison, fairly intact (*2, 3*). The progress of the disease is slowed by rearing the animal in the dark (*2*), yet after 150 days, even dark-reared animals show no measurable ERG (*4*). Noell and his colleagues were the first to show that axonal activity of single ganglion cells could be recorded from the optic tract of RCS rats even after the ERG had dissipated (*5*). Histology using glutaraldehyde fixation and thin plastic sections makes it clear that small numbers of nuclei of photoreceptor cells survive even in RCS animals as old as 2 years (*6*). These cells lack outer segments but appear to make synaptic contact with presumed bipolar and horizontal cell processes (*6*). Since visual pigment is housed mainly in the outer segments (*7*), these cells seem to be stripped of their fundamental ability to catch light quanta.

Behavioral measures reveal, however, that functional vision remains in 2-year-old RCS animals (*6, 8*). Can the remaining photoreceptor remnants, most of which are likely to be cone cells stripped of their outer segments (*6*), subserve vision in the aged RCS rat? We investigated the possibility that the spectral sensitivities of single ganglion cell axons recorded in the optic tract might indicate the surviving mechanisms mediating vision in the aged RCS rat.

We have found that the sensitivities of the ganglion cell axons recorded in the optic tract of RCS rats decline with age. Dark-adapted spectral sensitivities of single units recorded from RCS animals older than 5 months suggest a photopic mechanism in contrast to all dark-adapted ganglion cells in the normal animal, which show a scotopic spectral sensitivity. Histological studies corroborate this picture. The numbers of photoreceptor cells decline steadily with age, and the photoreceptor population changes in composition from predominantly rod (1.2 percent cones in the normal rat) to predominantly cone (73 percent cones at 197 days in the RCS rat).

Rats were anesthetized and paralyzed, and their respiration was controlled artificially. Blood pressure was monitored. A full eye ring provided further eye stabilization. The pupil was dilated, and a clear contact lens protected the eye (*9*). Single units in the optic tract were recorded with tungsten-wire-in-glass electrodes (Levick). The test light (150-W xenon arc lamp, Osram XBO 150 W/1) fully illuminated the surface of a diffusing Ping-Pong ball placed over the eye. This proved to be an effective way of maximally stimulating units with reduced sensitivities in RCS rats. Neutral-density and narrow-band interference filters, calibrated in the apparatus, were used in the test beam. The backgrounds were provided through a second channel illuminated by a 100-W solid tungsten lamp (General Electric). An on-line computer generated poststimulus time histograms. Luminances of test lights were adjusted to give criterion responses. Typically the impulse trains to ten presentations of the stimulus were combined to yield each histogram. A firing rate of five spikes per second above the

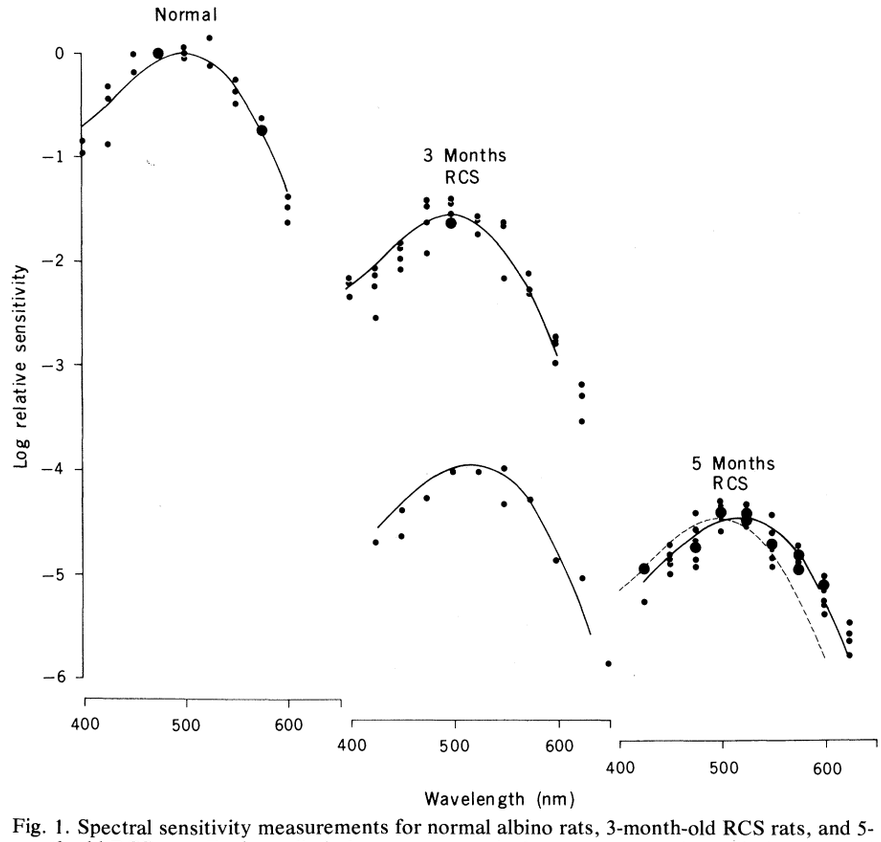

Fig. 1. Spectral sensitivity measurements for normal albino rats, 3-month-old RCS rats, and 5-month-old RCS rats. Each small circle represents a single measurement; large circles are used to represent multiple coincident determinations. The curves are derived from the Dartnall nomogram and have peaks of 500 or 520 nm chosen to best fit the data. The rhodopsin nomogram curve peaking at 500 nm fits the spectral sensitivity of units in the normal rat. At 3 months of age in the RCS rat, two classes of units were encountered. One class had spectral sensitivities matching the rhodopsin nomogram curve. The second class of units in this age group had spectral sensitivities showing an increased long wavelength sensitivity and matching the nomogram curve peaking at 520 nm. All units encountered and classified in the 5-month-old RCS rat showed photopic spectral sensitivities. The bulk of the units matched the nomogram curve peaking at 520 nm. The solid curve through the data for this age group is the nomogram curve peaking at 520 nm. The broken nomogram curve peaks at 500 nm and shows the consistent deviation from the rhodopsin template in these units.

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baseline rate was designated a threshold response. Straightforward and quick comparisons of the histograms gave reliable estimates of threshold. Seventeen pink-eyed, tan-hooded RCS rats ranging in age from 69 to 197 days and seven control albino Sprague-Dawley rats (herein called "normal") ranging in age from 84 to 300 days were studied (*10*). We now report on two age groups of RCS rats, those near 90 days of age and those near 150 days of age and older.

The relative sensitivity differences of units found in normal and RCS animals are shown in Fig. 1. For sensitivity to 500-nm light, near 90 days of age most units had thresholds elevated 1.56 log units above normal [standard error of the mean (S.E.M.), 0.17]. A small number of units recorded from animals at this age had thresholds elevated more than 2 log units above the majority of the RCS units (3.98 ± 0.49 S.E.M. with respect to the normal animals). Animals near 150 days of age or older had thresholds which were elevated 4.48 ± 0.09 log units above the normal.

In the normal rat, all units showed dark-adapted spectral sensitivities matching the rhodopsin nomogram curve peaking at 500 nm (Fig. 1) (*11*). At 3 months of age, 3/4 of the units recorded from RCS rats had spectral sensitivities that conformed to the rhodopsin nomogram curve. The few units at 3 months of age with dark-adapted thresholds 2 to 3 log units above the rhodopsin units had clearly photopic spectral sensitivities; they conformed to a nomogram curve peaking at 520 nm. At 5 months of age, all units encountered and classified showed photopic spectral sensitivities (*12*). The largest number of units at this age conformed to a single pigment nomogram curve with peak wavelength of 520 nm (Fig. 1).

Anatomical examination of the retinas from each of the three groups of animals supports the physiological findings (Fig. 2). Glutaraldehyde fixation, methylene blue staining, plastic embedding, and thin sectioning enhanced the visualization of the photoreceptor nuclei (*13*). The disappearance of photoreceptor outer segments appears to be complete by 197 days. There was also a progressive thinning of the outer nuclear layer. The number of photoreceptor nuclei per 1000-μm^2 area of the retina declined from 5.34 ± 0.24 rod nuclei and 1.92 ± 0.15 cone nuclei in the 86-day-old RCS rat to 0.64 ± 0.41 rod nuclei and 1.76 ± 0.29 cone nuclei in the 197-day-old RCS rat. These numbers contrast with the 225 ± 3.54 rod nuclei and 2.67 ± 0.27 cone nuclei in the normal albino at 99 days of age. Thus, there is a decline in the photoreceptor population and a concomitant change in its composition from predominantly rod (1.2 percent cones in the normal) to predominantly cone (73 percent cones at 197 days of age in the RCS rat).

Optic tract recordings of retinal ganglion cell activity thus show that a physiologically functioning postretinal visual pathway still exists in older RCS rats. Despite extensive degeneration of the photoreceptor layer, light-driven signals originating in the retina are passed on to higher centers by way of the optic tract. These signals could be the basis for behaviorally measured visual capacity in older RCS rats (*6*, *8*). We have shown that the spectral sensitivities of single ganglion cell axons recorded in the optic tract do not match that of rhodopsin, the visual pigment contained in rods, but rather implicate a cone photopigment as the basis for visual function. We have also shown a progressive change from a predominantly rod to a predominantly cone retina until, at 197 days in the dark-reared RCS rat, the only elements of the photoreceptor layer retained in near normal numbers are the cone cells, apparently devoid of outer segments (*14*). It is therefore possible that surviving cone remnants with a store of visual pigment (*15*) may be able to respond to light and effectively drive higher-order neurons.

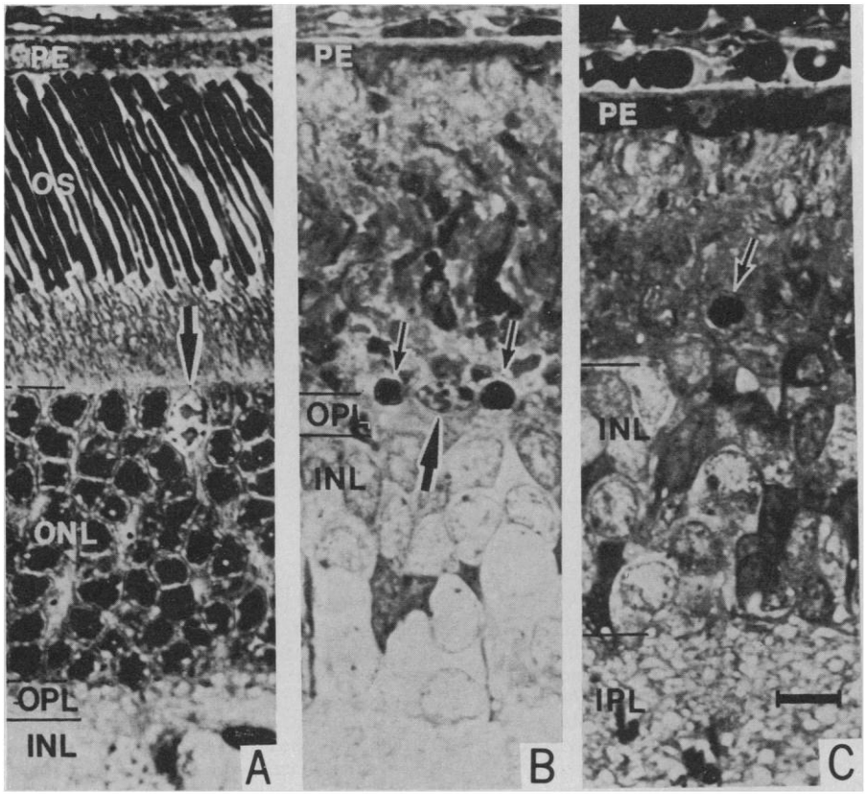

Fig. 2. Representative light micrographs of retinas from a 99-day-old normal albino rat (A) and RCS rats at 86 days (B) and 197 days (C). Abbreviations: *PE*, pigment epithelium; *OS*, outer segments; *ONL*, outer nuclear layer; *OPL*, outer plexiform layer; and *INL*, inner nuclear layer. Large arrows (in A and B) mark examples of nuclei in the ONL that were identified as cone nuclei; small arrows (in B and C) mark those identified as rod nuclei. Scale bar, 5 μm.

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References and Notes

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5. W. K. Noell, in *Retinitis Pigmentosa Clinical Implications of Current Research*, M. B. Landers, III, M. L. Wolbarscht, J. E. Dowling, A. M. Laties, Eds. (Plenum, New York, 1977), pp. 175–178.
6. M. M. LaVail, M. Sidman, R. Rausin, R. L. Sidman, *Vision Res.* **14**, 693 (1974).
7. Although visual pigments occur in highest concentrations in the photoreceptor outer seg-

ments, immunochemical studies in the frog [M M. Dewey, P. K. Davis, J. K. Blaise, L. Barr, *J. Mol. Biol.* **39**, 395 (1969)] showed that they also occur in isolated saccule membranes, in the myoid and ellipsoid of the photoreceptor inner segment, and in inclusions in the overlying retinal epithelium.

8. M. Kaitz, *Vision Res.* **16**, 151 (1976).
9. Animals had been reared in the dark since birth, with exposure to dim red illumination as necessary during changing of water bottles, and so forth. To maintain dark adaptation during surgery, a dim red light was used and, in addition, an opaque contact lens was temporarily placed over the experimental left eye. For further details of the procedures, see C. M. Cicerone and D. G. Green, *Vision Res.* **17**, 985 (1977).
10. The new congenic strain of RCS rats that is of the wild type at the retinal dystrophy genetic locus and is the best available genetic control for the pink-eyed, retinal dystrophic strain was not available to us at the time of this study. The control albino animals we used were free of the retinal dystrophy and hence are called "normal."
11. All data reported here were obtained under dark-adapted conditions. We have observed a Purkinje shift with light adaptation in the response of the normal rat ganglion cell axon (C. M. Cicerone, in preparation).
12. Certain units recorded from animals near 5 months of age were so insensitive that determining spectral sensitivity was not possible, for light was lost when narrow-band interference filters were inserted. Other units were best fit by nomogram curves with peaks different from 500 or 520 nm. Although the histology showed remaining rod nuclei (which we carefully tried to distinguish from pycnotic nuclei), none of the units encountered and classified at 5 months of age showed a rhodopsin spectral sensitivity. We are led to two alternatives. (i) The rhodopsin units may have been those too insensitive to allow a determination of spectral sensitivity. (ii) The process of light transduction by rods and cones may differ so that, despite the loss of outer segments, cones may be capable of responding to light, but rods without outer segments may be totally incapacitated.
13. Eyes were enucleated, lentectomized, and immersed in a fixative containing 2 percent formaldehyde, 3 percent gluteraldehyde, 1 percent acrolein, and 2.5 percent dimethyl sulfoxide in a 0.1*M* sodium cacodylate buffer at *p*H 7.2. The tissue was postfixed in 2 percent osmium tetroxide in the same buffer, strained in 0.5 percent uranyl acetate in maleate buffer of *p*H 5.8, dehydrated through alcohols, and embedded in Epon. Sections 1 μm thick stained with 0.1 percent methylene blue in 1 percent borax were used for all light microscopy. In normal animals, the cone nuclei were easily identified by their larger size, lighter nucleoplasm, position in the outer third of the outer nuclear layer, and characteristically clumped chromatin (*6*). Identification was more difficult in the RCS animals. Some nuclei exhibited the distinctively clumped pattern and lighter nucleoplasm of the normal animals but were somewhat smaller than normal cone nuclei. Other nuclei were microglia-like, and still others were smaller and contained a single mass of darkly staining chromatin within the nuclear membrane. This last class of nuclei resemble rod nuclei but also may be a stage in the degeneration of either rod or cone nuclei. Only the conelike chromatin patterns were counted as cone nuclei for this report. Microglia-like nuclei were not reported. The third class were counted as rodlike, but their identification is tentative. In any event, the numbers reported are the upper limit for rodlike nuclei in the RCS animals.
14. Anatomical [L. D. Carter-Dawson, M. M. LaVail, R. L. Sidman, *Invest. Ophthalmol. Visual Sci.* **17**, 489 (1978)] and physiological [U. C. Dräger and D. H. Hubel, *J. Comp. Neurol.* **180**, 85 (1978)] research on the visual system of mice with hereditary retinal degeneration has been reported. These studies are closely related to our work in the RCS rat and point to a similar pattern of visual decline in mice with hereditary retinal degeneration.
15. W. Noell has suggested that the plasma membrane of cone cells may house visual pigment and that this store of pigment is the basis for visual capacity in the aged RCS eye.
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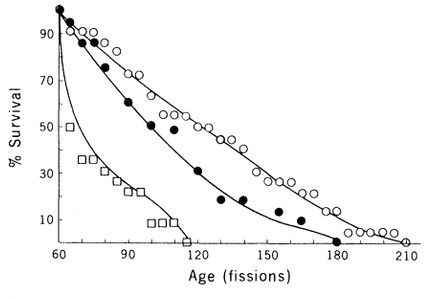
DNA Repair and Longevity Assurance in *Paramecium tetraurelia*

Abstract. *At given doses and clonal ages, ultraviolet irradiation-induced DNA damage reduced clonal life-span, but when followed by photoreactivation, extension of clonal life-span was observed. If photoreactivation preceded the ultraviolet treatment, no significant beneficial effect was detected. Because studies of others have shown that photoreactivation repair monomerizes the ultraviolet-induced cyclobutane dimers in DNA, but does not affect the other photoproducts, these results indicate that DNA damage can influence the duration of clonal life-span unless that damage is repaired. Repeated treatment with ultraviolet and photoreactivation resulted in significant mean and maximal clonal life-span extension when compared with untreated controls, and it is assumed that the rejuvenation effect was due to the correction or prevention of some age damage.*

Paramecia were used to study the biological effect of ultraviolet-induced DNA damage versus photoreactivation (PR)-repaired damage on clonal senescence. These cells exhibit cellular aging (*1*), show age-correlated sensitivity to ultraviolet reversible by PR (*2*), have been shown to monomerize induced dimers by PR in their nuclear DNA (*3*), express age-induced mutations (*4, 5*) suggesting loss of repair with increased age, and have many parallels with human cells in culture (*4, 6*). Clonal senescence can be characterized by a decreased probability that a given cell will give rise to a viable cell at the next cell division (*1, 7*). As in multicellular organisms, fertilization marks the origin of a new generation, and predictable changes occur in the phenotypes of cells (*1, 2*). Death of the clone occurs some 150 to 200 cell divisions, or fissions, later—in about 40 days, when the procedures described below are used.

Aging cells were maintained in daily isolation lines (*8*). Replicate samples of the fertilized cells were carried as sublines. Each day, one cell of a subline was passed seriatim to a new depression; its products were counted on the following day, and the daily fission rate was determined. A subline is considered dead when an isolated cell disappears. The fission age of the cell is the number of fissions since fertilization. The mean clonal life-span is the average fission age at death of all sublines. Maximal life-span is the largest fission age observed for any subline of a clone at death. Isolation lines provide the source of cells for the controls, treatment with ultraviolet only, ultraviolet plus PR, PR plus ultraviolet, and PR only (*9, 10*).

As the fission age of the clone increased, the ultraviolet dose required to reduce the mean clonal life-span decreased; 5400 ergs at cells 40 fissions old versus 2700 ergs at cells 140 fissions old (Table 1). At critical doses and ages, a negative shift in the survival curve was



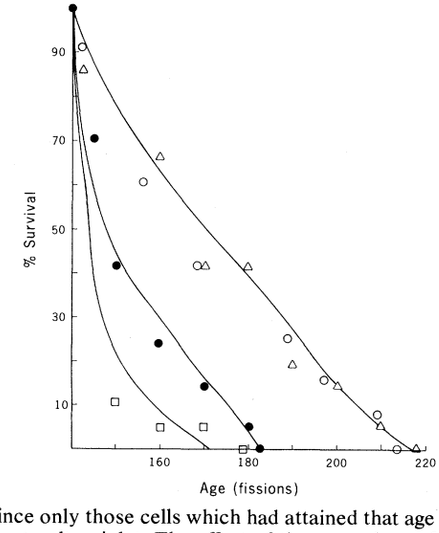


Fig. 1 (left). Effect of ultraviolet only and ultraviolet plus photoreactivation on clonal life-span. At 60 fissions, one member of a dividing cell was given ultraviolet only at 5400 erg/mm^2 (squares), the other cell member was given ultraviolet plus photoreactivation (open circles). Control cells (closed circles) were taken from the same population. The ultraviolet treatment was given 1^1/$_2$ hours after cell division. The experiment starts at 100 percent survival since only those cells which had attained that age were used. Fig. 2 (right). Induced resistance to ultraviolet. The effect of the same dose of ultraviolet (2700 erg/mm^2) on clonal life-span varied when cells 140 fissions old were previously untreated (open squares) or had received ultraviolet plus photoreactivation when 80 fissions old (open triangles). The respective untreated controls (closed circles) and control cells which received ultraviolet plus photoreactivation when 80 fissions old (open circles) are included.

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