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"Lighting Conditions and Retinal Development in Goldfish:
Photoreceptor Number and Structure"

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# Lighting Conditions and Retinal Development in Goldfish: Photoreceptor Number and Structure

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**The retinas of 63 goldfish were examined after varying durations of exposure to one of three environmental lighting conditions beginning before hatching: constant light (340 lux), cyclic light (12 hr 320 lux, 12 hr dark) and constant dark. Up to 8 months, no effects of constant light or dark on photoreceptor numbers or structure were apparent. Densities of rod and cone nuclei were normal and all retinal layers appeared normal by light microscopy. Exposure to constant light for 12 months or longer resulted in a reduction in rod density by 37%. Cone numbers were unaffected by constant light, even with exposures of 3 yr, and rod and cone outer segments were normal in length at 11–20 months under all environmental conditions. Due to poor survival, only one animal was available for quantitative examination from the group reared in constant dark 12 months or longer. Photoreceptor size and number in this retina were similar to those in the constant light condition. The results suggest that the formation and maturation of rods and cones in goldfish retina is unaffected by rearing in constant light. However, long-term exposures (≥12 months) may disrupt maintenance of differentiated rods. Invest Ophthalmol Vis Sci 29:27–36, 1988**

The nature of the visual environment influences many aspects of visual structure and function. One of the most profound of these interactions is the deleterious effect of exposing photoreceptors to constant illumination over a period of days.[1-4] Even low (<1000 lux) to moderate (1000 to 3000 lux) levels of illumination typical of a normal photopic environment can cause damage, the severity of which varies in different species.[1] The mechanism of light damage is not known, but it is thought to be mediated by absorption of photons by photoreceptors as part of the normal process of visual transduction.[5,6]

Retinal damage by constant light has been demonstrated in adult rodents[7,8] primates,[9] frogs[10] and fish,[11] among others. In general, photoreceptors are the most affected retinal cells, with the first evidence of damage being loss of outer segments.[7,8] Less severe lesions, such as damage to or loss of outer segments, are reversible, but if the process is allowed to continue, photoreceptors eventually die, and in mammals this loss of cells is irreversible. Rods appear to be more sensitive than cones, which persist longer in damaging lighting conditions.[12,13] A few reports describe damage and cell loss in the inner retinal layers as well as in photoreceptors.[10,11]

In contrast to the large literature on light damage, the effects of constant darkness on retinal structure are not well studied. A few reports deal with development of the retina and differentiation of photoreceptors in constant darkness, but the conclusions are inconsistent. Eakin[14] reports that in tadpoles (*Hyla*) the photoreceptors differentiate normally in constant darkness, whereas Besharse and Brandon[15] found that in cave salamanders, in which degeneration of photoreceptors occurs normally at the end of larval development, photoreceptor loss was more severe in animals raised in constant dark than in constant light. Hollyfield et al[10] also found greater cell loss in adult frogs (*Rana*) kept up to 20 days in constant dark compared to animals kept in constant light.

All of these studies have sought to determine the influence of the visual environment on the differentiation or maintenance of retinal cells. A separate question is whether alterations in the visual environment can modify the initial formation, by cell division, of retinal neurons, and especially photoreceptors. This question is difficult to answer for mammals

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because most retinal cells are born prenatally.[16,17] It can be addressed in teleost fish, where retinal neurogenesis continues throughout larval and adult life.[18,19] In many fish, rods continue to accumulate as the retina grows throughout postembryonic life. Specialized progenitor cells, scattered across the retina within the layer of rod nuclei, undergo repeated mitotic divisions and produce new rods that are inserted into the photoreceptor mosaic.[19-22] It is not known if these new rods are differentially vulnerable to damage by the visual environment.

To study the effects of the lighting environment on retinal neurogenesis, we raised goldfish from hatching through 3 years of age in constant light, constant dark, or cyclic light. Our results demonstrate that neurogenesis in the postembryonic retina is apparently unaffected by lighting conditions, and that teleost photoreceptors are remarkably resistant to the damaging effects of constant light or constant dark.

## Materials and Methods

All procedures adhered to the ARVO Resolution on the Use of Animals in Research. Mature goldfish obtained from Ozark Fisheries (Stoutland, MO) were spawned in the laboratory.[23] At the beginning of the experiment, about 2 days prior to hatch, the embryos were placed into their respective lighting environments in 10 gallon aquaria with approximately 20 embryos per tank. Embryos and fish were maintained at about 20°C in aerated tap water. Larvae and young juvenile fish were fed live nauplii of *Artemia* (brine shrimp) until they reached an approximate size of 1 cm (standard body length, exclusive of tail), at which time a dry commercial goldfish food (Tetramin) was given. In general, fish were fed once daily.

Fish were removed from the tanks at intervals of 1 week to 36 months after hatching, and their retinas were processed for histology as described below. The experiment was repeated twice with minor variations in histological and morphometric procedures (Series 1 and Series 2, below). The results of the two replications were virtually identical, so the data were combined for analysis.

### Environmental Lighting Conditions and Number of Animals

The cyclic lighting (LD) group was kept in daylight fluorescent lighting (Sylvania F40/D), turned off between 8 PM and 8 AM, CST. Light intensity at the water's surface was 320 lux (86 $\mu$W/cm$^2$); measured through a column of water equivalent to the 10 gal tanks, it was 310 lux (83 $\mu$W/cm$^2$). The group maintained in continuous illumination (LL) was in a separate room, with daylight fluorescent lighting of 340 lux (91 $\mu$W/cm$^2$) at the water's surface. The third group of animals was kept in continuous darkness (DD) in a lightproof cabinet in a photographic darkroom. These fish were fed using a dim red (Kodak Wratten filter no. 29; Rochester, NY) flashlight.

Sixty-three retinas were examined: 20 from fish that had been reared in LD, 32 from fish reared in LL and 11 from fish reared in DD. Survival rates for DD fish were low compared to the other two groups. The poor survival rate combined with the impossibility of counting photoreceptor nuclei in some DD retinas (see *Results*) reduced the total number of DD fish available for photoreceptor counts to five. Even though this number is small, the data from these fish are included in the results to indicate trends observed with different durations of darkness during rearing.

Because we lacked animals in the LD group at survival times of >12 months, we have included for comparison three fish purchased as juveniles from the same supplier that provided our breeding stock. The lens diameters of these fish were comparable to LL and DD fish at >12 months, but they were of unknown age (see Table 1). These fish had been hatched and grown in outdoor ponds and therefore experienced a cyclic lighting environment prior to arrival in the lab. Upon receipt they were placed in conditions similar to the LD group. Histological and morphometric procedures were the same as for the experimental animals. Table 1 lists the 42 fish for which we have quantitative data on photoreceptor numbers, along with their lens diameters.

### Histological and Morphometric Procedures

*Series 1:* Embryos were from a single spawn in May, 1981. Up to three animals were removed from each experimental group at 1, 2, 4, 8, 12, 26 and 52 weeks after hatching. The retinas of two additional LL fish from this series were processed at 36 months of age.

Fish <4 weeks of age were fixed whole in 2% glutaraldehyde, 2% paraformaldehyde. Older animals were anesthetized (Finquel, Ayerst, New York, NY) and decapitated before fixing; this procedure was completed within 1 min. The corneas were punctured and the tissue was fixed overnight. DD fish were sacrificed under dim red illumination. Tissues were dehydrated and embedded in Epon 812, either as intact heads (≤4 weeks old) or eyes (≥8 weeks old). Sections were cut at 1 $\mu$m thickness and stained with methylene blue-azure II.

The initial analysis was performed without knowledge of the light exposure history of the retinas. The lens diameter was measured from a camera lucida tracing of its circumference in the section in which the diameter was maximal. Measurements were corrected for histological shrinkage of 15%. This value was determined by comparing the diameters of three eyes measured after fixation and measured again after embedding. Comparison with the same measures made before fixation showed that the fixation itself caused negligible shrinkage.



Cone and rod nuclei were identified based on cytological features described previously.[18,19,23] Briefly, cone nuclei form a single row along the external limiting membrane. They are larger and paler stained than rod nuclei. Rod nuclei are smaller, darker and stacked in rows up to three or four deep, vitread to the cones. For cell counts, we selected three nonadjacent meridional sections. Within each section, we counted the number of cone and rod nuclei in a segment of retina 0.4 mm long superior to the optic disc. Counts were made with a ×100 oil immersion objective. The means of the three samples were computed and expressed as planimetric densities (number per mm$^2$), corrected for counting errors due to split nuclei with a modified Abercrombie factor.[24]

*Series 2:* The experiment was replicated with a second group of fish from a single spawn in February, 1983. A maximum of two fish were removed from each of the three lighting conditions at 1, 2, 8 and 11 months; one fish in DD was sacrificed after 20 months, two in DD after 25 months, and two in LL after 36 months. The four fish at 25 months or longer were used for the companion psychophysical study prior to sacrifice.[23]

Tissues were fixed as in Series 1, except that 0.1% picric acid was included in the fixative in some cases. After 2 to 3 days in fixative, tissues were rinsed in buffer. Eyes were dissected from the larger fish (≥8 months old), the lens was removed and its diameter measured with calipers. Lens diameters for younger fish were measured from sections and corrected for shrinkage as described in Series 1. Tissues were dehydrated to 95% ethanol and embedded in glycolmethacrylate (Sorvall Embedding Medium, Dupont, Newtown, CT). Sections were cut at 3 $\mu$m thickness and every other slide was bleached in potassium permanganate/oxalic acid[19] to decolorize melanin in the retinal pigmented epithelium (RPE). Sections were stained with Lee's mixture of methylene blue and pararosaniline.[19]

Only retinas from animals in this series 8 months or older were used for cell counts, but 1 and 2 months retinas were examined qualitatively. For the cell counts, two meridional sections were selected, and cone and rod nuclei were counted in three retinal segments, each 0.1 mm in length, chosen from the central one-third of the retina, for a total of six samples from each eye. Planimetric cell densities were computed and corrected for split nuclei as in Series 1.

**Table 1.** List of experimental animals on which morphomotric measurements were made

| *Fish* | *Condition* | *Age (months)* | *Lens diam (mm)* |
|---|---|---|---|
| 1A1 | LD | 0.25 | 0.08 |
| 1A4 | LD | 0.25 | 0.09 |
| 1C3 | LL | 0.25 | 0.17 |
| 1C1 | LL | 0.25 | 0.18 |
| 2B5 | LD | 0.5 | 0.19 |
| 2D3 | LL | 0.5 | 0.10 |
| 2D1 | LL | 0.5 | 0.18 |
| 4C2 | LD | 1 | 0.23 |
| 4A3 | LL | 1 | 0.30 |
| 4A4 | LL | 1 | 0.34 |
| 8A1 | LD | 2 | 0.69 |
| 8D1 | LL | 2 | 0.82 |
| 8D4 | LL | 2 | 0.86 |
| 12B2 | LD | 3 | 0.69 |
| 12A4 | LL | 3 | 0.70 |
| 12A1 | LL | 3 | 0.88 |
| 12C4 | DD | 3 | 0.55 |
| 12C2 | DD | 3 | 0.68 |
| 26C1 | LD | 6 | 0.98 |
| 26C3 | LD | 6 | 1.11 |
| 26A2 | LL | 6 | 1.03 |
| 26A4 | LL | 6 | 1.03 |
| 26B2 | LL | 6 | 1.13 |
| 26D6 | DD | 6 | 0.98 |
| 8M2 | LD | 8 | 1.25 |
| 8M6 | LL | 8 | 1.07 |
| 8M5 | LL | 8 | 1.30 |
| 8M8 | DD | 8 | 1.50 |
| 11M7 | LD | 11 | 1.49 |
| 11M5 | LL | 11 | 1.16 |
| 11M6 | LL | 11 | 1.37 |
| 52A2 | LD | 12 | 1.29 |
| 52A3 | LD | 12 | 1.33 |
| 52C4 | LL | 12 | 1.40 |
| 52C3 | LL | 12 | 1.42 |
| 52C2 | LL | 12 | 1.14 |
| 20M1 | DD | 20 | 1.35 |
| 12AB1 | LL | 36 | 1.94 |
| 4AB1 | LL | 36 | 2.38 |
| 0NC1 | LD | ? | 1.70 |
| 0NC3 | LD | ? | 2.70 |
| 0NC4 | LD | ? | 2.80 |

Each fish is identified by a code (first column). The lighting condi under which it was raised (cyclic, LD; constant light, LL; constant dark, D is indicated in the second column. The age of the animal at sacrifice months) and the diameter of its lens (in mm) are given. The last th animals (0NC1, 0NC3, 0NC4) were purchased as juveniles, and their a are unknown (see text).

The lengths of cone and rod outer segments w measured in five fish from Series 2 with exposu times of 11 or 20 months. Care was taken to ensu that only intact outer segments, contained co pletely within the 3 $\mu$m thickness of the section, we selected for measurement.[23] Bleached sections we used for measurements because photoreceptor ou segments in unbleached sections were partially c scured by overlying melanin granules.[23] Thirty more outer segments of three morphological ty (rods, long double or single cones and short sin cones) were selected from each retina and were dra with a camera lucida at a final magnification

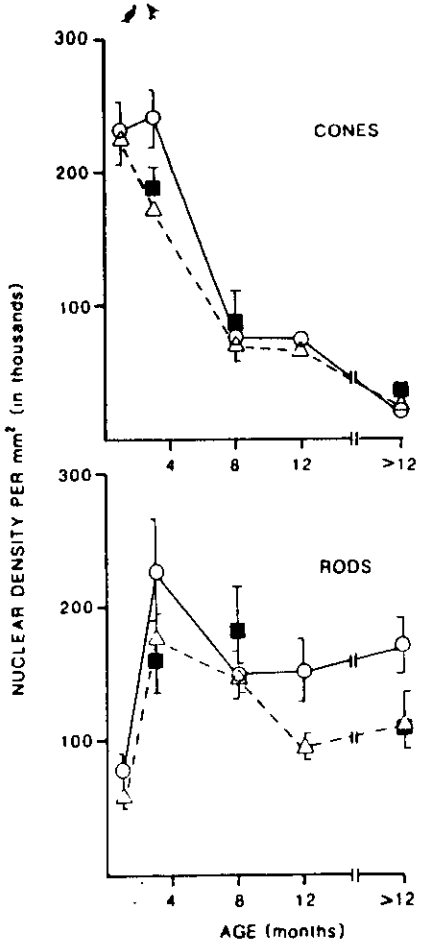


Fig. 1. Photoreceptor densities as a function of age. Cones are not affected by rearing in LL or DD; but rod density is reduced significantly after 12 months. Circles, LD; triangles, LL; filled squares, DD. Each point represents the mean of two to six retinas, with the exception of the DD value at >12 mo., which is a single retina (see Table 2). Error bars are one standard error of the mean, bars for LD point leftward, those for LL point rightward and those for DD point both left and right. The data for the LD condition are connected by solid lines, and those for LL are connected by broken lines; DD data are unconnected. The value of the X-coordinate for each point is arbitrarily set at the upper end of the range for that bin (see Table 2).

×1150. Mean lengths of outer segments were determined with the aid of a Zeiss (New York, NY) IBAS image analysis system.

## Results

This experiment was designed to measure the effects on photoreceptor densities of rearing in different lighting conditions. The results will be presented first in those terms. It is important to recognize that in normal goldfish, photoreceptor density is correlated more strongly with the size of the animal (or the size of its eye) than the age.[18] We have therefore also examined the data as a function of the size of the fish at the time of sacrifice.

### Photoreceptor Densities Related to Age and Length of Exposure

Figure 1 shows rod and cone densities in goldfish exposed from hatching to ages of 1–36 months in constant light (LL), constant dark (DD) or cyclic light (LD). Table 2 shows the number of fish contributing to each point in Figure 1.

Cone density decreases with age in normal goldfish due to growth of the eye and stretching of the retina.[18,19] Cone densities in the LD fish decreased with age (Fig. 1). Rearing in LL or DD did not affect this pattern (Fig. 1). At any given age, cone densities in fish reared in LL or DD were equivalent to those in fish reared in LD, with the exception of the 2–3 month group where densities in LD fish were higher. This discrepancy is the result of variations in the size of the fish in this sample, due to variability in individual growth rates (see below).

A previous study[19] showed that the age profile of rod densities in normal fish is different from that of cones. During larval stages (up to 3 weeks after hatch) and in young juveniles the density of rod nuclei increases rapidly until it reaches a peak at 2–4 months. During this period, rods are added centrally by mitotic division and subsequent differentiation of special rod precursor cells scattered across the retina; this does not occur for cones.[19,21,22] Rod proliferation in the young retina is of sufficient magnitude to surpass the opposing tendency, stretching, which pulls apart cones and other cells. Between 4 and 8 months, proliferation of rod precursors wanes, and rod density thereafter remains approximately constant.

The LD animals in the present experiment followed this pattern. Up to 8 months the same pattern of rod addition occurred in LL and DD animals, but at 12 months and beyond differences became apparent. In the LD (control) group, rod density remained stable from 8 to >12 months, but in the LL group rod density continued to fall until 12 months, when it stabilized at a value 37% lower than in the LD fish.

The effect of DD on rod density is less clear because fewer fish survived in this condition and because most retinas from fish exposed for long durations had severe disruptions that precluded quantifying photoreceptor densities (see below). Only one retina could be used for photoreceptor counts from the DD group reared 12 months or longer. The density of rods in this fish's retina fell within the range of values for the LL animals, suggesting that rod densities might also be reduced by rearing in DD.



**Table 2.** Photoreceptor densities by age

| *Age (months)* | *Condition* | *N* | *Mean cones per mm² (thousands)* | *SEM* | *Mean rods per mm² (thousands)* | *SEM* |
|---|---|---|---|---|---|---|
| ≤1 | LD | 3 | 228 | 23 | 78 | 13 |
| | LL | 6 | 224 | 4 | 58 | 8 |
| 2–3 | LD | 2 | 239 | 22 | 227 | 37 |
| | LL | 4 | 172 | 6 | 175 | 20 |
| | DD | 2 | 188 | 15 | 158 | 24 |
| 6–8 | LD | 3 | 77 | 15 | 148 | 18 |
| | LL | 5 | 71 | 13 | 146 | 11 |
| | DD | 2 | 87 | 24 | 182 | 33 |
| 11–12 | LD | 4 | 75 | 6 | 151 | 23 |
| | LL | 5 | 66 | 4 | 94 | 10 |
| >12 | LD | 3 | 20 | 2 | 170 | 21 |
| | LL | 2 | 22 | 1 | 111 | 20 |
| | DD | 1 | 36 | — | 109 | — |

Fish are grouped into five bins according to their age at sacrifice, and further subdivided by experimental condition (LD, LL, DD). The number of fish (N), the mean densities of cones and rods per mm² (in thousands) and the standard error of the mean (SEM) are given. Rod densities were significantly lower in LL retinas than in LD retinas ($P < 0.025$, one-tailed rank sum test[44]; $P < 0.001$, $\chi^2$ goodness-of-fit test). Cone densities did not differ with experimental condition. Statistical tests were not attempted with DD data.

### Can Differences in Growth Rate Account For the Differences in Rod Density?

It is possible that development in general could be slowed by rearing in unusual lighting environments. This issue is important to consider because in goldfish the number of retinal cells is more closely related to body size than to age.[18] If, for example, fish reared in constant light grew at slower rates than fish reared in cyclic light, then a reduction in rod density in older fish could be a reflection of smaller eye size instead of a direct effect of constant light on photoreceptor development. Figure 2 shows that this was not the case.

Each point on Figure 2 represents the diameter of the lens for one fish, as a function of the fish's age. Lens diameter has been used as an index of growth because eye size is more closely related to retinal parameters than to body length.[18] Figure 2 shows that no systematic differences in growth rate occurred with these experimental conditions. Logarithmic curves fit by least squares regression (see Figure caption) indicate that the three groups do not differ from each other. Differences in growth rates among LL,

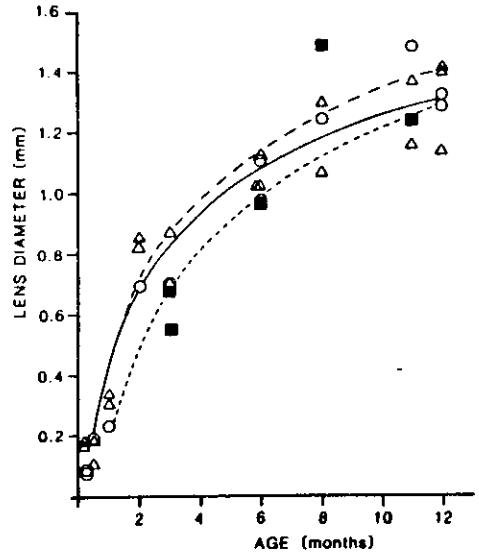


Fig. 2. Growth of fish in different lighting conditions. This graph shows lens diameter as a function of age for fish up to 12 months old, from Table 1. Each point represents an individual fish. An extra point has been added for a DD fish at 11 months; this fish does not appear in Table 1. Circles, LD; triangles, LL; filled circles, DD. An exponential function was fit by least squares to the data from each experimental condition ($r = 0.98$ for LD, solid curve; $r = 0.95$ for LL, upper dashed curve; $r = 0.84$ for DD, lower curve). No differences in growth rate were apparent for fish in the different rearing conditions.

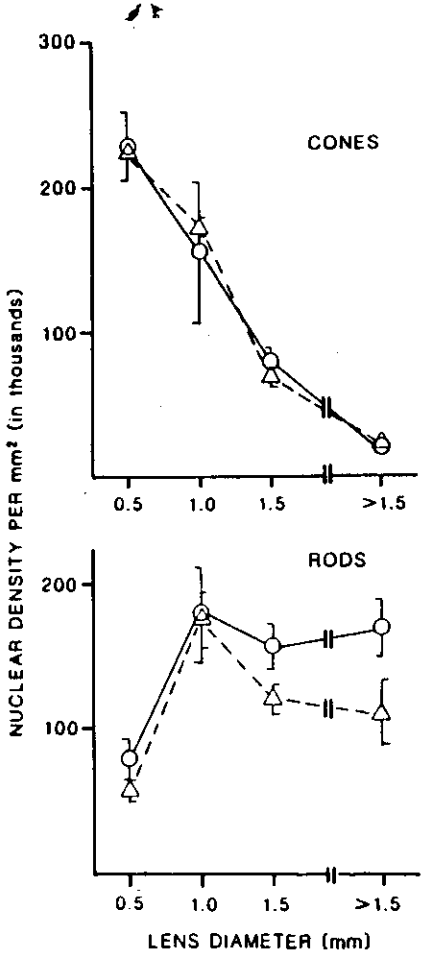


Fig. 3. Photoreceptor densities as a function of size. When differences in growth rate are factored out, the effect of rearing in LL on rods (but not cones) is still apparent. Symbols and conventions as in Figure 1. DD data omitted for clarity. Values listed in Table 3.

DD and LD fish therefore cannot account for the differences observed in photoreceptor densities.

Another factor to consider is that goldfish normally grow at different rates; fish of the same age in Figure 2 differed in lens diameters by as much as a factor of 2, even when reared in LD. This variation in individual growth rate contributes to the variance in photoreceptor density when plotted as a function of age. Its contribution can be factored out by plotting photoreceptor density as a function of lens diameter, which, as Figure 2 shows, was not affected by the experimental lighting conditions.

In Figure 3 we have plotted the density of cones and rods as a function of lens diameter, with the data grouped in bins (see caption and Table 3). For the cones this representation of the data gives a smoother curve than the plot by age (Fig. 1). At no lens diameter was there any difference in cone density between LL and LD fish. Moreover, for both groups the decrease in cone density was directly proportional to lens diameter, as would be expected if the decrease in density were due to expansion or stretching of the retina. For the rods as well, the curves are quite similar in shape to those of Figure 1. Rod densities are lower in LL fish compared to LD fish, by 24% and 35%, respectively, in the two largest size classes. We conclude that rod densities are reduced by rearing in constant light, while cone densities are not.

### Outer Segment Lengths

When measured after 1–2 years of exposure to constant light or constant dark, rod and cone outer segment lengths were not different from LD controls (Fig. 4). The length of rod outer segments, averaged over all three lighting conditions, was 34 μm; long cones (containing red- or green-sensitive photopigment[26,27]) averaged 14 μm and short cones (containing blue-sensitive photopigment[26,27]) were just under 7 μm.

### Additional Observations on Retinal Structure

We made qualitative observations on 19 retinas in addition to those listed in Table 1. These fish were maintained under one of the specified lighting conditions for 1–2 months (n = 5 in LD, four in LL and three in DD), 11 months (n = 1 in DD) or 2–3 yr (n = 4 in LL and 2 in DD). The retinas from the 1–2 month old fish appeared normal and similar to each other under all three lighting conditions. There was a severe loss of rods in the 11 month DD retina. Because this retina was so different from all the others, it was not included in the quantitative analysis. In four out of nine of the 2–3 yr retinas (three in Table 1, plus the six mentioned here: four LL and two DD) the laminar arrangement of the photoreceptors was grossly distorted; no such effect was ever observed in retinas exposed for 12 months or less. The disruption included scalloping of the outer nuclear layer and the layer of photoreceptor cell processes, and it occurred in two LL and two DD retinas. Because of the distortion and folding in the outer layer in these retinas it was not possible to obtain histological sections strictly



Table 3. Photoreceptor densities by size

| Lens diameter (mm) | Condition | N | Mean cones per mm² (thousands) | SEM | Mean rods per mm² (thousands) | SE |
|---|---|---|---|---|---|---|
| ≤0.5 | LD | 3 | 228 | 23 | 79 | 1 |
| | LL | 6 | 224 | 4 | 58 | 1 |
| 0.51–1.0 | LD | 4 | 156 | 49 | 179 | 3 |
| | LL | 4 | 172 | 6 | 175 | 2 |
| | DD | 3 | 162 | 27 | 177 | 2 |
| 1.1–1.5 | LD | 5 | 79 | 9 | 157 | 1 |
| | LL | 10 | 68 | 6 | 120 | 1 |
| | DD | 2 | 49 | 13 | 129 | 2 |
| >1.5 | LD | 3 | 20 | 2 | 170 | 2 |
| | LL | 2 | 22 | 1 | 111 | 2 |

Fish are grouped into four bins according to their lens diameter (in mm), and further subdivided by experimental condition (LD, LL, DD). The number of fish (N), the mean densities of cones and rods per mm² (in thousands) and the standard error of the mean (SEM) are given. Again, a rank sum test[44] of the difference between LL and LD fish shows that overall there were fe rods in the LL condition ($P < 0.025$) and a $\chi^2$ test shows that the distributi of rod densities with age is different between LL and LD fish ($P < 0.001$); fish have fewer rods.

perpendicular to the layer of photoreceptors; the section plane passed obliquely through many of the cells. Therefore we could not measure planimetric densities of photoreceptors in these preparations, although both rods and cones appeared to be present. Because none of the LD fish from the spawns of Series 1 and 2 remained at 2–3 yr for comparison, we also cannot be certain that the retinal disorganization was due to the experimental lighting conditions and not due to unknown, but non-light related conditions. However, the fact that about half of the LL and DD retinas from 2–3-yr-old fish had normal histological organization argues against such factors.

## Discussion

We have shown that rearing goldfish in constant light leads to a 30 to 40% loss of rod nuclei in the outer nuclear layer, but only after exposures of more than 8 months. Our data tentatively suggest that constant darkness may similarly lead to rod loss. In contrast to rods, the cone numbers were unaffected by any experimental condition. Because lens diameters were also normal after rearing in constant light, we conclude that retinal growth and the initial production of new photoreceptors in the young goldfish is independent of environmental input.

One of the principal goals of this study was to determine the role of visual stimulation in retinal neurogenesis and development, and we selected the goldfish as an experimental animal because photoreceptor addition occurs postembryonically. In fact, over 95% of the retinal surface area in a 2-yr-old goldfish is composed of neurons generated postembryonically and added as annuli of new retina at the peripheral margin in the freely-swimming, visually functional animal.[28] Only a small circular patch near the cent of the retina, accounting for 5% of the total are contains neurons that were postmitotic at hatchin At hatching the outer nuclear layer contains on cones[19] and therefore virtually all of the rods are ge erated postembryonically. A period of vigorous pr liferation by special rod precursor cells during tl first few months after hatching leads to a rapid acc mulation of rods,[19,22] and our results show that th process is independent of lighting environment. A though we have not quantified retinal neurons oth than photoreceptors, the normal appearance of tl cellular layers in retinas from LL and DD fish, cor bined with the lack of an effect on number of cone suggests that postembryonic neurogenesis of all re

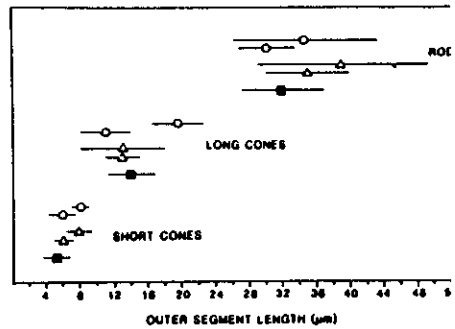


Fig. 4. Length of photoreceptor outer segments in differe lighting conditions. No consistent effect of rearing in LL or DD w observed on either cone or rod OS length after 11–20 month Symbols as in other figures. Each point is the mean of 30 or mo measurements of individual photoreceptors from one retina; err bars are one standard deviation.

nal neurons is similarly independent of visual stimulation.

This conclusion is consistent with the general notion that the initial establishment of neuronal populations is accomplished according to predetermined genetic instructions.[29,30] In most species, however, neurogenesis is primarily an embryonic event.[29,30] Although mammalian retinas show limited neurogenesis after birth,[16] vision is not fully developed at this stage. Fish retinas, in contrast, continue to generate neurons while in a fully functional state. The only comparable situation in mammals is the olfactory epithelium, where the population of sensory cells is continuously renewed.[31] But the olfactory neurons are a special case in that they normally die and are replaced by new cells, whereas neurons in the fish retina do not normally die, so that continued cell proliferation serves to increase their total numbers. We have demonstrated that despite their potentially vulnerable position, the neuronal germinal cells in the fish retina continue to produce new neurons and these new cells continue to differentiate, even when the visual environment is abnormal.

Exposing *adult* fish to constant light can have a marked and rapid effect on the retina. Penn[11] placed golden shiners (*Notemigonus*) in constant light of 850 or 1250 lux (2.5–3.7 × higher than the intensities we used) for up to 14 days and then measured several indices of retina structure, including rod outer segment length and number of cells in the outer and inner nuclear layers and the ganglion cell layer. He found a decrease in cell density in all layers and a reduction in length of rod outer segments after exposures of 4–14 d, compared to controls kept in cyclic light. The damage was greater in dorsal retina compared to ventral. Rapp and Williams[8] similarly found greater cell loss in the dorsal retina of rats exposed to constant light. This regional variation in severity of light damage has been attributed to a higher concentration of rhodopsin in dorsal retina due to either longer outer segments or more numerous rods in that region.[8,11] This interpretation is based on the premise that light damage is directly related to the amount of light absorbed by the photopigment.[5,6] Regional variations in rod loss were not investigated systematically in the present study.

Marotte et al[32] kept juvenile and adult goldfish (3.5–8.0 cm body length) in constant light of 1–2 footlamberts for up to 9 months. This intensity is about 1/10 of ours, which was 18 footlamberts at water surface (M. Powers, personal observations). They found a 15 to 30% decrease in thickness of the outer nuclear layer in the constant light condition, with the greater effects in the larger fish. Although they did not count individual nuclei, they interpreted this result as a loss of rods, compatible with results of the present study. When rod loss was first observed here, the fish were nearly equivalent in size to the smallest ones used by Marotte et al (about 3 cm in body length), and by the end of our study, the fish were about 8 cm, ie, equivalent to their largest animals.

The index used here to examine the rod population (cell density) represents the net product of addition and loss. We observed a net loss of rods in fish reared in constant light, beginning after nearly a year of exposure. This decrease in rod density probably represents cell death rather than lack of rod addition, for the following reasons. If the nature of the visual environment had influenced rod genesis, we would have expected to see some sign of this during the early postembryonic period when the majority of rods are generated. It is possible that constant light destroys *differentiated* rods throughout the period of exposure, even though a net effect is not seen until 1 year. In the youngest fish, dividing rod precursor cells may be sufficiently numerous and/or responsive to increase their rate of production of new rods so as to counteract the cell loss. We have independent evidence that the rate of proliferation of rod precursors can be regulated by other extrinsic factors, such as nutrition (P. Raymond, unpublished observations). In older fish, rod addition normally diminishes,[19] and at this stage the rod precursor pool may not be capable of overcoming the cell loss induced by constant environmental lighting conditions, and as a result the density of rods declines. It is known that when adult fish are placed into constant light, photoreceptors are lost within a few weeks.[11,32] Clearly in this case cell death is involved, as the length of time is too short for any effect on cell addition to be manifest. To summarize, photoreceptors are certainly destroyed by constant light in older fish, and this may also occur in younger fish, but in the latter increased production of rods could compensate for the loss. The observation by Marotte et al[32] that larger fish suffered more severe loss of rods fits with this interpretation. From our present data we could not assess the rate of rod production, nor did we make a concerted search for pyknotic, dying cells, so we offer this suggestion as a hypothesis that remains to be tested.

There are a few other developmental studies involving constant light in lower vertebrates,[14,33,34] and all of the studies, including the present one, indicate that constant light in the young retina does not produce damage comparable to that seen in adults, whether in frogs[10] or in fish.[11] The situation is therefore similar to that in rats, in which light damage is greater in adults than in young animals.[35] The reason



for this is not entirely clear, but in rodents it may be related to hormonal changes accompanying puberty. We do not know whether the loss of rods observed in the goldfish retina, beginning at about 1 year of age, was coincident with the onset of sexual maturity. It is possible, since goldfish have been reported to spawn at 1 year of age.[36]

## Outer Segment Lengths

Retinal photoreceptors in goldfish exhibit a rhythmic daily shedding of the tips of their outer segments: cones shed at light offset and into the dark period and rods shed at light onset.[37,38] Shedding in most species is abolished during the first few days in constant light, but in *Rana* after 20 days of constant light, shedding occurs spontaneously and sporadically.[10] In amphibians[39] and rats[40] it has been shown that under conditions in which rod outer segment shedding is inhibited, ie, constant light, addition of new membranous discs at the base of the outer segments continues, and the net effect of this imbalance is that the outer segments increase in length. This also occurs in goldfish kept in constant light of 340 lux for 7 days.[41,42] Under more intense illumination, however, rod outer segments may actually decrease in length after 4–14 days.[11] Presumably the net loss of outer segment material in constant light of moderate intensities involves an increase in the amount shed, rather than a decrease in the rate of assembly of new outer segment discs, because Besharse et al[43] have shown that light actually *increases* the rate of disc assembly in several species of amphibians, including larval and adult forms. Our observation that the lengths of cone and rod outer segments in goldfish reared for 1 to 2 years in either constant light or constant dark were equivalent to those in cyclic light implies that after prolonged exposure during development, a balance is achieved between disc assembly and shedding, independent of lighting condition.

In summary, the goldfish retina grows normally, adding neurons and differentiating, in the presence of constant light or constant dark for almost 1 year after hatching. With continued exposure, however, a partial loss of rod nuclei is observed in constant light, and perhaps also in constant dark. No effect on number of cones or on the lengths of cone or rod outer segments was found, even with exposures from hatching up to 3 years of age. We conclude that constant environmental lighting conditions interfere with the maintenance of functional rods, but not with the production of new rods.

**Key words:** photoreceptors, constant light, retinal development, rods, cones

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