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VISUAL DETECTION BY THE ROD SYSTEM IN GOLDFISH OF DIFFERENT SIZES

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Abstract—New rods are continually generated and inserted across the entire differentiated retina in juvenile and adult goldfish; no other retinal cells share this characteristic. How does the preferential addition of rods affect visual function? To examine the relation between continued rod addition and visual sensitivity, we measured absolute threshold in fish of different sizes. Twenty-nine fish were trained in a classical conditioning paradigm, and psychometric functions were obtained for each of them for detection of a 532 nm light 5 sec in duration, 140 deg in angular subtense, presented while the fish was fully dark adapted. We found that absolute threshold (expressed in terms of retinal photon density) was lower in larger fish, but by a very small amount: on average, large fish (15.4 ± 0.5 cm standard body length) were 1.45 times more sensitive than small fish (4.3 ± 0.3 cm). Morphometric analysis showed that the planimetric density of rods in goldfish retina increases at a similar rate between small and large fish, while the density of retinal ganglion cells declines between small and large fish (by a factor of 3.8). The ratio of rods to ganglion cells (a possible indicator of neural convergence) increased, but by a factor that is too large to reconcile with the psychophysical results (5.3 ×). The results suggest that absolute visual threshold in the goldfish is closely related to the density of rods in the retina.

Retina Scotopic sensitivity Neural development Rods Goldfish

INTRODUCTION

Many teleost fishes continue to grow during adulthood, and part of the growth involves the addition of new neurons. Changes with age in the number of neurons and synapses in the visual pathway have been documented in several species (Muller, 1952; Lyall, 1957; Johns and Easter, 1977; Kock and Reuter, 1978; Fisher and Easter, 1979; Johns and Fernald, 1981), and some of the relations between these natural neuronal changes and visual physiology (Macy, 1981; Macy and Easter, 1981; Branchek, 1984) and behavior (Baerends *et al.*, 1960; Hester, 1968; Hariston *et al.*, 1982) have been examined.

For the most part, previous studies of visual function during development have been carried out on light-adapted preparations. Yet one of the most striking phenomena of retinal growth in teleosts is the disproportionate addition of rods (Muller, 1952; Lyall, 1957; Johns and Easter, 1977; Sandy and Blaxter, 1980; Johns, 1982). In the goldfish, *Carassius auratus*, all new neurons (except rods) are born at the retinal margin, forming concentric annuli like the growth rings of a tree (Johns, 1977; Johns and Easter, 1977; Rusoff and Easter, 1980). The retina also grows by stretching or expansion, resulting in a lower density of retinal neurons in larger eyes (Muller, 1952; Ali, 1964; Johns and Easter, 1977; Johns, 1977; Kock, 1982). In contrast to other retinal neurons, new rods are generated *throughout* the retina from special precursor cells lying among the already differentiated rods in the outer nuclear layer (Johns, 1982; Raymond and Rivlin, 1987). The addition of new rods from dividing precursors is apparently sufficient to counteract the decrease in rod density due to stretch, because the planimetric density of rods (per mm^2 of retina) does not decrease during adult life (Johns and Easter, 1977).

It has been postulated that the function of the preferential addition of rods to the fish's retina is to maintain scotopic sensitivity constant as the fish eye grows (Johns and Fernald, 1981), because the probability that a given photon entering the eye will encounter a rod would be constant with constant planimetric density of rods. This hypothesis assumes that the length of the rod outer segments is constant in adult fish, which is approximately true (Raymond, 1985). But other interpretations are plausible as well. For example, if scotopic sensitivity depends on

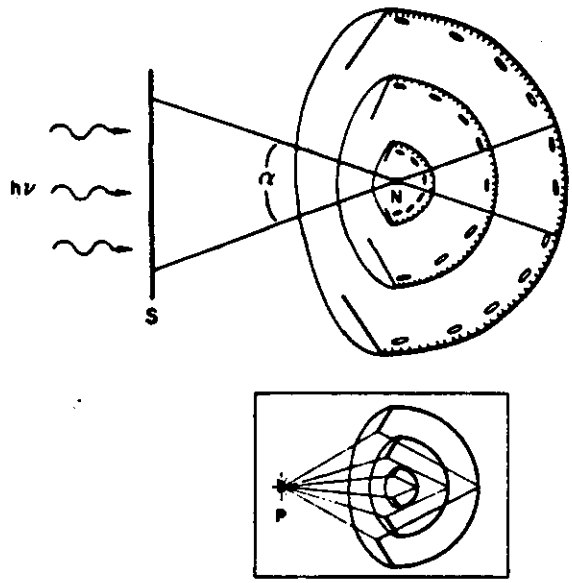

Fig. 1. Schematic drawing of goldfish retinas from three different sizes of fish (from parameters given in Easter *et al.*, 1977). Top: because of the precise scaling of the eye with growth, a visual stimulus of subtense α at plane *S* relative to the nodal point of the eye, *N*, provides photons to progressively larger areas of retina as the eye enlarges. The number of retinal cells increases with growth (see Johns and Easter, 1977); shown here are relative numbers of rods (tick-marks) and ganglion cells (ellipses), approximately to scale for the change in retinal sizes illustrated. Note that the same visual stimulus covers more rods and ganglion cells as the eye enlarges. Bottom: the pupil enlarges with growth, allowing more photons from a given visual stimulus to enter the eye in larger fish.

the amount of convergence between rods and neurons in the proximal retina (Otten, 1981), and if the number of synaptic connections made by the new rods onto higher-order cells also increases (Kock and Stell, 1985), then scotopic sensitivity might be expected to increase as the fish grows.

In this and the following paper (Falzett *et al.*, 1988) we exploit the unique qualities of the goldfish retina to begin an inquiry into the neural determinants of absolute threshold. In particular, we describe correlations between psychophysical measurements of absolute visual threshold and the natural, growth-related alterations in the neuronal population of the adult goldfish retina. Measurements were made on dark-adapted fish under conditions known to favor detection by the rod system (Powers and Easter, 1978). To simplify the comparison of thresholds from fish of different sizes, we used a stimulus that subtended the same visual angle throughout.

Figure 1 illustrates some of the changes that occur with growth in goldfish retina, and shows how the neuronal population that receives a stimulus of constant visual angle changes as a result. In the top part of the figure retinas from 3 sizes of fish are represented schematically, with small tick-marks indicating relative numbers of rods and ellipses representing relative numbers of retinal ganglion cells in each retina. Notice that a stimulus of constant visual angle α at plane *S* would provide photons to a progressively larger retinal area as the fish grows. Within that area the planimetric density of rods changes very little, while the density of ganglion cells decreases. This means that as the fish grows the ratio of rods to ganglion cells in its retina—a possible indicator of the amount of convergence within the scotopic system—is continually increasing, and might imply that larger fish should be substantially more sensitive to dim lights than smaller fish.

The bottom part of Fig. 1 illustrates another property of the goldfish eye that also points to a possible increase in sensitivity as the fish grows: the pupil enlarges, allowing more light from any given point on the stimulus to enter the eye. Thus, the retinas of larger fish would receive more photons than those of smaller fish from a stimulus of constant irradiance.

METHODS

Common goldfish (*Carassius auratus*) were obtained from commercial suppliers (Ozark Fisheries, Stoutland, Mo. or Grassyforks Fisheries, Grassyforks, Md). They were classified according to standard body length (sbl), measured from nose to base of tail: small (3.1–5.3 cm), medium (7.6–11.0 cm) and large (12.5–19.1 cm). Eight fish were used for the morphometric measurements (2 small, 2 medium, 4 large), and 29 fish were used in the psychophysical experiments (10 small, 10 medium, 9 large). Fish were maintained under environmental light and temperature regimes designed to minimize any intrusion of rhodopsin (see Tsin and Beatty, 1979).

The psychophysical measurements were carried out in 2 separate experiments because the changes we observed in the first study were small and we were concerned that they could have resulted from individual differences not related to retinal parameters. As will be shown below, the results of the 2 replications were similar; they were also consistent with the results of a third study (not reported here) in

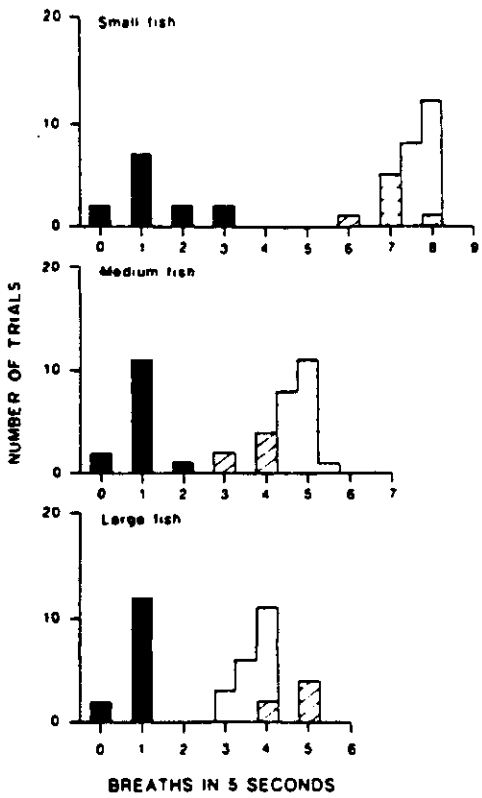

Fig. 2. Histograms of respiration rates during single test sessions for a small, a medium and a large fish. The number of breaths in 5 sec is shown for every trial, with pre-stimulus values indicated by open bars and rates during presentation of the stimulus indicated by shaded and hatched bars. The shaded bars show rates that were scored as a "response," while the hatched bars show rates that were not sufficiently different from pre-stimulus values to be counted as a response. Regardless of the baseline rate, which was considerably faster for small fish, all animals tended to produce only 1 respiratory movement when a "response" was scored.

which slightly different stimulus conditions were used (Powers and Bassi, 1981).

Psychophysical procedures

Fish were restrained in an aquarium so that the right eye faced a rear projection screen, and absolute visual threshold was measured for each fish by means of a classically conditioned response suppression technique described in detail before (Powers and Easter, 1978; see also Northmore and Yager, 1975). Each fish was conditioned to withhold breathing when a monochromatic stimulus 5 sec in duration (the CS) was followed by a mild electrical shock to the tail (the US); current was adjusted within sessions to deliver the minimum that reliably produced suppression of respiratory movements. A "response" was said to occur if the respiration rate during the stimulus interval was $\leq 50\%$ baseline respiration rate, as calculated during six 5 sec intervals preceding onset of the visual stimulus. Respiration movements were recorded with a glass bead thermistor placed near the animal's mouth (Powers and Easter, 1978).

Respiration rate varies with body length in teleost fishes, with smaller fish having faster rates (Fry, 1957). To ensure that our criterion for response would produce comparable data for all sizes of fish used in this experiment, we compared the statistics of breathing rate samples from small, medium and large fish during single test sessions (to be described below). Three such samples, from well-trained fish, appear in Fig. 2. As expected, baseline respiration rates (open bars) varied considerably: the rate for the small fish in this example was about twice that of the large fish. When visual stimuli were presented however, fish of every size tended to produce either very few respiratory movements (dark bars) or a larger number of movements that was not reliably different from baseline rate (hatched bars). The difference between the hatched and dark bars in Fig. 2 is that the rates represented by dark bars were scored by the experimenter as representing "responses" (relative to the statistics of the baseline rate on the trial of interest), while those represented by hatched bars were not. We therefore consider the response criterion to be approximately equal for all sizes of fish.

Stimuli subtended 140 deg visual angle and were presented on a totally dark background following at least 1 hr dark adaptation. To maintain the visual angle constant for different sizes of fish, we measured the distance from the eye to the rear-projection screen and computed the appropriate diameter (in cm) needed to produce 140 degrees. Small and medium fish were positioned within the apparatus so that the stimulus subtended 140°. For large fish the final aperture (see Fig. 1 in Powers and Easter, 1978) was adjusted to obtain that diameter; this adjustment was taken into account in subsequent calculations of retinal flux. Small head movements could occur in this apparatus, even though fish were restrained. Such movements could have altered the angular subtense of the stimulus by $\pm 20\%$ (Powers and Easter, 1978). To account for the threshold changes reported below, larger fish would have had to remain systematically closer to the stimulus screen throughout all tests, which is highly unlikely.

Stimulus wavelength and intensity were controlled by interference (Perkin Elmer) and neu-

tral density (Melles Griot) filters placed in the collimated portion of a beam from a quartz–halogen source (Powers and Easter, 1978). Inter-stimulus intervals were generally 0.5–1.5 min. The highest intensity stimulus used in this experiment was 3.5 log units above absolute threshold for the rod system, as measured previously in medium-sized fish under similar conditions (Powers and Easter, 1978).

After a fish had been trained (defined as responding to $\geq 8/10$ trials in two successive training sessions), psychometric functions were obtained in a staircase procedure (Powers and Easter, 1978) at 532 nm. The reported data are based on two separate test sessions per fish, with 20 trials per test session. 532 nm was chosen because it is near the peak of the absorption spectrum of goldfish rod porphyropsin (Schwanzara, 1967) and because previously existing evidence suggested that dark-adapted threshold at 532 nm reflects the action of rods in this species (Powers and Easter, 1978).

Stimulus irradiance at threshold (in photons $cm^{-1} sec^{-2}$ at the cornea) was computed from measurements made with a calibrated photodiode (PIN-10DFP, United Detector Technology) placed at the plane of the pupil. Retinal flux values were derived for fish of different sizes by taking into account the area of the pupil (Falzett, 1984), the optical density of the eye media (Bassi *et al.*, 1984) and the area of the stimulus on the retina (Powers and Easter, 1978).

Morphometric measurements

Although a previous study (Johns and Easter, 1977) quantified retinal cell densities in goldfish of approximately the same sizes as those used here, we felt it necessary to repeat these measurements because counts of optic nerve fibers in goldfish gave lower estimates of total ganglion cell number (Easter *et al.*, 1981) and because the largest fish used for the psychophysical measurements reported here were larger than the largest fish used for morphometric analysis in the earlier work (Johns and Easter, 1977). Accordingly, in the present study, improved histological techniques were used (plastic rather than paraffin embedment) and more stringent criteria were applied to identify ganglion cells in a larger range of body lengths.

Twelve eyes from 8 goldfish were used for the determination of retinal cell densities. Fish were decapitated, eyes were removed rapidly, the cornea was slit and the lens was removed. Eyecups were fixed overnight in buffered 1–3% gluteraldehyde, 1–3% paraformaldehyde and (in some cases) 1% picric acid. Tissues were dehydrated to 95% ethanol, embedded in Sorvall Embedding Medium (Dupont), sectioned at 3 μm and stained with methylene blue–pararosaniline (Johns, 1982).

For rod counts, one meridional section was selected from each eye, and 4–6 sampling regions, spaced equally along the linear extent of the retina from one ciliary margin to the other, were identified. All of the rod nuclei contained within a 100 μm length in each sampling region were counted. For ganglion cell counts, cell nuclei identified as ganglion cells on the basis of cytological features (large round, pale nucleus with a rim of basophilic cytoplasm) were counted in three meridional sections from each eye. Care was taken to exclude presumed glial cells with oblong nuclei, similar in size to ganglion cell nuclei; these glia may have been counted as ganglion cells in the previous study (Johns and Easter, 1977). We believe that these are not ganglion cells because retrograde transport of HRP (applied to the cut optic nerve) does not label them (Raymond, unpublished observations).

Cell counts were corrected using a modified Abercrombie correction factor (Konigsmark, 1970). Histological shrinkage was estimated at 15%, independent of size of the eye (Raymond *et al.*, 1987); counts have been adjusted accordingly. The mean densities of rod and ganglion cell nuclei ($\#/cm^2$) were calculated from measurements for each eye, and the data are reported for both eyes from a given fish, when both were counted. After counting rods and ganglion cells in the first 7 eyes, it became clear that ganglion cell densities and rod:ganglion cell ratios did not correlate with the trends in the psychophysical data, whereas rod densities did. Hence for the last 5 eyes we counted only rods.

RESULTS

Absolute threshold and size of fish

Examples of psychometric functions from fish of different sizes appear in Fig. 3. The slopes of functions from fish in this study were similar to previous measurements on medium-sized fish (Powers and Easter, 1978) and did not differ among small, medium and large categories. The number of trials needed to train fish in the

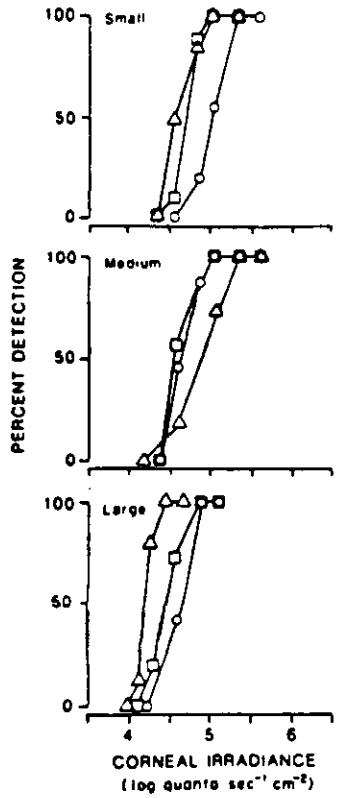

Fig. 3. Sample psychometric functions from 3 small, 3 medium and 3 large goldfish. Examples were selected to illustrate the range of sensitivities; all other functions in a given size category fell within the extremes shown here. The percent of trials on which a given fish responded is given as a function of the absolute intensity of the stimulus, as measured at the cornea. No difference in slope was observed with size of fish. Threshold was defined as the intensity where the probability of detection was 0.5.

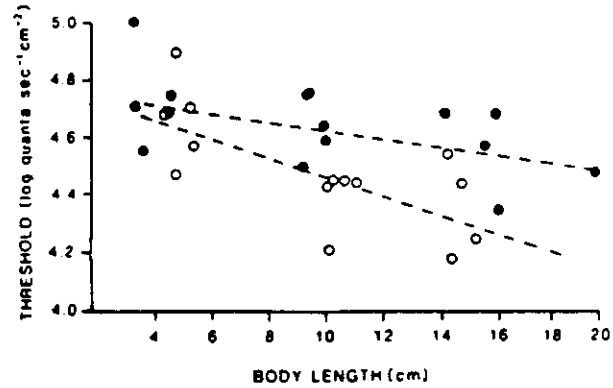

Fig. 4. Absolute visual threshold for 29 fish of different sizes. Threshold irradiance at the cornea is plotted as a function of standard body length for each subject. Open and solid points represent the results of different experiments; threshold decreased significantly with body length in both experiments by approximately the same amount. The two lines represent the least squares regression equations for each experiment ($T_c = -0.015\,\mathrm{sbl} + 4.79$, $r = 0.54$, $P < 0.05$ for solid circles; $T_c = -0.033\,\mathrm{sbl} + 4.79$, $r = 0.70$, $P < 0.01$ for open circles). The slopes of both lines are significantly different from zero ($t = 2.311$, d.f. = 13, $P < 0.05$ for solid circles; $t = 3.960$, d.f. = 12, $P < 0.01$ for open circles). A 2 (replication) × 3 (fish size) factorial analysis of variance showed that the mean threshold for all sizes differed in the 2 replications ($F_{1,23} = 11.50$, $P < 0.005$) but that small, medium and large fish had different thresholds in both replications ($F_{2,23} = 7.66$; $P < 0.005$). There was no interaction between replication and fish size ($F_{2,23} = 1.64$, $P < 0.25$).

classical conditioning task also did not differ with body length.

Figure 4 shows absolute threshold measurements for fish of different sizes at 532 nm, expressed in units of photon density at the cornea. Each point represents the quantal irradiance required for detection with $P = 0.5$ for an individual fish, derived from the 50% point of its psychometric function (Fig. 3). The solid and open circles are data from two replications. In both experiments the range of variability across fish within a given size category was 0.3–0.5 log unit, as in Powers and Easter (1978) and did not vary with body length. Moreover the threshold values for medium sized fish were virtually identical, on average, to those found previously for fish of this size (Powers and Easter, 1978).

A trend toward lower thresholds with increasing body length is apparent in Fig. 4. The trend was statistically significant in both replications by linear regression analysis as well as by analysis of variance (see figure legend for details). The slopes of the best-fitting lines drawn in Fig. 4 show that threshold decreased by about 0.03 log unit per cm body length in one experiment, and by about 0.02 log unit per cm in the other. These slopes were statistically indistinguishable ($t = 1.709$, d.f. = 25, $P < 0.1$; see Howell, 1987).

Because the data from both experiments were similar, we combined them to yield an overall equation that relates the absolute visual threshold of the rod system to body length. That relation is illustrated in Fig. 5, where the mean rod threshold ±2 SEM for all fish in each size category has been plotted along with the regression equation

$$T_c = -0.021\,\mathrm{sbl} + 4.77 \qquad (1)$$

where T_c = absolute threshold, in log quanta $\mathrm{sec}^{-1}\,\mathrm{cm}^{-2}$ incident *at the cornea*, and sbl = standard body length in cm, nose to base of tail.

By this equation, the expected change in threshold between a 4 cm fish and a 16 cm fish would be 0.25 log unit.

Table 1. Threshold and morphometric measurements for small and large goldfish

	N	Body length in cm ($\bar{x} \pm$ SEM)	Measured value per cm^2 of retina
Small fish			
Retinal irradiance at threshold	10	4.3 ± 0.25	$8.7 \pm 1.0 \times 10^3$ photons/sec
Rod density	3	3.8 ± 0.37	$12.1 \pm 0.87 \times 10^6$ rods
Ganglion cell density	2	3.4 ± 0	$4.4 \pm 0.74 \times 10^5$ ganglion cells
Mean rod: ganglion cell ratio	2	3.4 ± 0	31:1
Large fish			
Retinal irradiance at threshold	9	15.4 ± 0.51	$6.1 \pm 0.79 \times 10^3$ photons/sec
Rod density	6	13.7 ± 0.38	$17.0 \pm 1.7 \times 10^6$ rods
Ganglion cell density	3	12.8 ± 0.17	$1.2 \pm 0.15 \times 10^5$ ganglion cells
Mean rod: ganglion cell ratio	3	12.8 ± 0.17	158:1

Numeric values for the average of N fish are shown for small and large size categories. Retinal irradiance at threshold was determined psychophysically; all other measurements were made from histological material. Ganglion cell densities were determined in only some of the retinas in which rods were counted. A rod:ganglion cell ratio was computed for each eye where both were counted, and the mean ratios were calculated from these values. The ratios of rods to ganglion cells were 23.4 and 37.8 for small fish and 121.7, 129.3 and 222.7 for large fish. Individual values of all morphometric measurements are plotted on a relative logarithmic scale in Fig. 6.

The retinal stimulus at absolute threshold

The relation between the *retinal* stimulus and body length differs from equation (1) because pupil diameter and focal length change with growth in fish (Charman and Tucker, 1973; Easter *et al.*, 1977; Fernald and Wright, 1983; Falzett, 1984), and because the eye media absorbs photons (Bassi *et al.*, 1984). At 532 nm the absorption by the media is similar in all sizes of goldfish, so this factor can be considered constant. Pupil diameter in goldfish (Falzett, 1984) grows more slowly than focal length (computed from lens diameters in Falzett, 1984, using the relation focal length = 2.36 × lens radius found by Easter *et al.*, 1977). This means that the numerical aperture decreases slightly with growth. Taking all these factors into account and computing the least-squares regression equation for retinal irradiance at threshold (T_r) yields

$$T_r = -0.015\,\text{sbl} + 3.96. \quad (2)$$

Note that the retinal irradiance (T_r) required for threshold decreased by a factor of about 1.5 (0.18 log unit) between 4 and 16 cm body length, while the corneal irradiance (T_c) decreased by about a factor of 2 (0.25 log unit). Thus, considered either at the cornea or at the retina, absolute threshold changed only slightly with increasing body length.

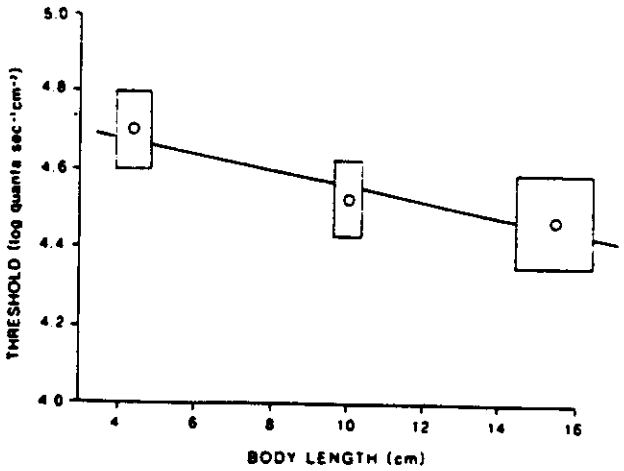

Fig. 5. Summary of the relation between absolute threshold and standard body length in the goldfish. The points are the mean log corneal irradiance for small, medium and large fish, from the data in Fig. 4. The boxes show ±2 SEM for threshold and for body length. The solid line is the best fit by least squares linear regression analysis ($T_c = -0.021$ sbl $+ 4.77$, $r = 0.52$, $P < 0.01$). Threshold decreased by 0.02 log unit per cm body length.

Comparison to morphometric measurements

Table 1 shows results of the cell counts for small and large fish, together with the average retinal irradiance required for threshold detection by small and large fish. Mean rod density increased by a factor of 1.4 between small and large fish, while ganglion cell density decreased by a factor of 3.7. We computed rod-to-ganglion cell ratios from the cell counts, including only those eyes for which both cell types had been counted. For the small fish in Table 1 the mean rod–ganglion cell ratio was 31:1; the mean for large fish was 158:1. Thus, as expected from previous measures, rod density increased slightly with size—0.14 log unit, on average, between small and large fish—while ganglion cell density decreased by a larger factor—0.57 log unit in the present set of measurements. And the ratio of rods to ganglion cells, which may be taken as a possible indicator of retinal

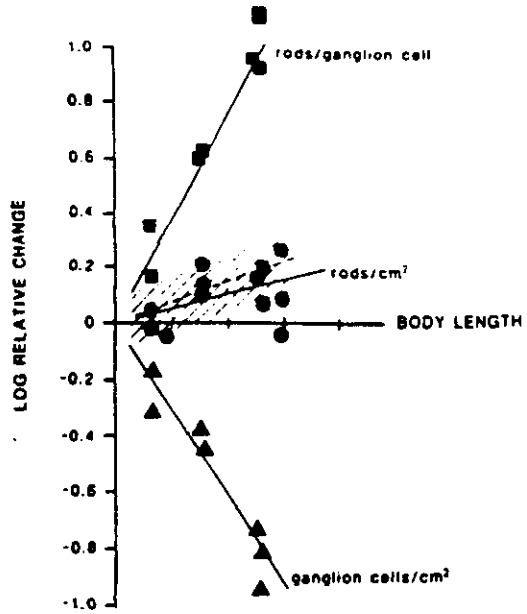

Fig. 6. Comparison of absolute sensitivity, density of rods, ganglion cells, and rod: ganglion cell ratio. See Methods for details concerning cell counts, and Table 2 for the slope of each function. To facilitate comparison, log relative increase or decrease in a given parameter is shown as a function of body length. Each mark on the abscissa indicates a 5 cm increment in standard body length. The reciprocal of absolute threshold (sensitivity), expressed in units of retinal irradiance ($-T_r$), is represented by the dashed line; the range of ± 2 SEM is indicated by shading. Note that only rod density (circles) has a similar rate of change to that of visual sensitivity. Neither the density of ganglion cells (triangles) nor the nominal amount of convergence of rods onto higher-order cells (squares) appears to be related to absolute sensitivity. Linear regression lines have been drawn through each data set. The lines show the following relationships, adjusted vertically on the log axis to coincide at zero: log rods $cm^{-2} = 0.0092\,sbl + 7.10$ ($r = 0.40$, d.f. = 11, NS); log ganglion cells $cm^{-2} = -0.063\,sbl + 3.89$ ($r = 0.95$, d.f. = 6, $P < 0.01$); log rods:ganglion cell = $0.074\,sbl + 1.18$ ($r = 0.98$, d.f. = 5, $P < 0.01$).

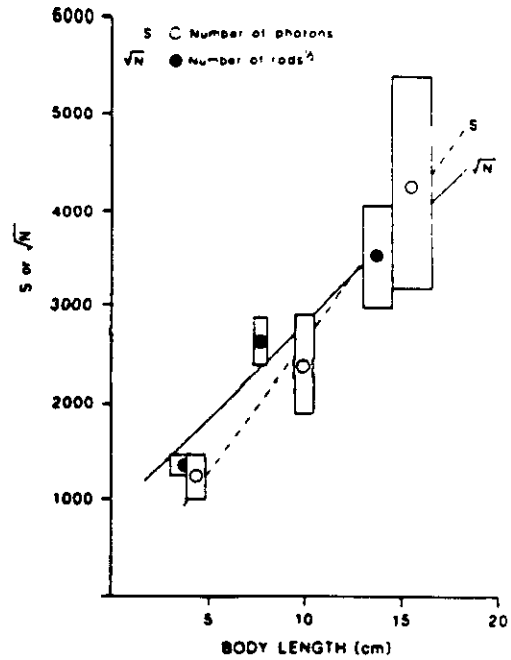

Fig. 7. Because the pupil enlarges with growth (see Fig. 1) the retinas of larger fish receive more photons at threshold. This figure shows the relation between the photon flux at threshold (open symbols) and the square root of the number of rods covered by the retinal stimulus (solid symbols).

convergence, increased by 0.71 log unit—more than a factor of 5.

The results of the cell counts for all sizes of fish are shown graphically in Fig. 6, along with a range of values that represents absolute sensitivity at different body lengths, expressed in retinal terms ($-T_r$). All values are expressed logarithmically in this figure to facilitate comparison among the rates of change of the various factors with increasing body length. The curves are least squares regression lines calculated from the logarithms of each set of data, constrained to pass through the origin; the actual values of the slopes are in Table 2.

It is clear that neither the density of ganglion cells in the retina nor the rod:ganglion cell ratio changed with body length in the same way absolute sensitivity did. Note that of the three morphological measures taken in this study, the change in density of rods correlates best with the change in density of photons incident at the retina at psychophysical absolute threshold.

DISCUSSION

Neural correlates of absolute threshold

The absolute visual threshold of the goldfish becomes lower with increasing body length. The

Table 2. Changes in various retinal and psychophysical properties with growth

Property	Slope (log/cm sbl)
Psychophysical threshold	
Retinal irradiance	0.015
Retinal flux	0.047
Corneal irradiance	0.021
Retinal morphology	
Density of rods	0.009
√Number of rods	0.036
Number of rods	0.071
Rods per ganglion cell	0.074

Relative change in psychophysical threshold and retinal composition with growth. The match is best between retinal irradiance at threshold and the planimetric density of rods in the retina. Relative change is expressed as the logarithmic slope of the function relating the property of interest to standard body length. Sources for each value are: retinal irradiance equation (2), retinal flux Fig. 7, corneal irradiance equation (1), rod density and rods per ganglion cell Fig. 6, number of rods and square root number of rods Fig. 7.

magnitude of the change is small but statistically significant. Table 2 lists the rates of change of threshold and of the various retinal properties measured in this study. It illustrates, again, that the change in threshold is not easily accounted for by changes in the rod: ganglion cell ratio. Nor are the results of the present experiment compatible with a model that relates changes in psychophysical threshold to changes in the photon sensitivity of *individual* rods, because the low quantum–rod ratios reported here, for all sizes of fish, are similar to a previous report (Powers and Easter, 1978) in which we demonstrated that psychophysical threshold is reached when individual rods receive $\leqslant 1$ quantum.

The role of photoreceptors

Of the anatomical parameters measured in this experiment, the density of rods per unit area of retina correlated best with absolute threshold. As the goldfish grows and its sensitivity to light increases, the number of rods covered by any given angular subtense increases, and these two factors maintained a nearly constant relationship throughout the range of sizes we tested. The close relationship between threshold retinal irradiance and planimetric density implies a primary role for rod photoreceptor density in determining absolute visual threshold. This inference is supported by data from a separate series of experiments in which goldfish were reared in constant light (Powers *et al.*, 1987; Raymond *et al.*, 1987). In such fish, at $\geqslant 12$ months of age, the planimetric density of rods is reduced by 30–40% and psychophysical absolute threshold is elevated by a comparable amount. Together with the present results, these studies suggest that the preferential addition of rods to the continually stretching retina in normal goldfish serves to maintain photon-catching ability relatively constant during growth.

The planimetric density of rods sets an initial limit for absolute threshold, in the sense that it determines the probability that a photon incident on the retina will encounter a rod. We have recently shown that another property of the rods—the length of the outer segment (ROS)—is also an important determinant of absolute threshold: Goldfish kept in constant light for 1 week have elongated ROS and concomitantly lower thresholds than goldfish kept in cyclic light (Bassi and Powers, 1986). In terms of rod-related parameters, then, planimetric density and outer segment length are both closely related to psychophysical detection at absolute threshold.

Does "noise" increase with growth? There are more rods in larger goldfish retinas, both in terms of absolute numbers (Johns and Easter, 1977) and number per degree visual angle (see Fig. 1). If each rod contributes to the "noise" against which a signal must be detected, then the amount of noise in the retina should increase, at least at the level of the input to second order cells. For at least these cells, one would expect the threshold signal to increase as noise does. In Fig. 7 we plot a hypothetical "noise" function, where noise is considered to be proportional to $\sqrt{N}$ and N is the number of rods in the retinal stimulus field (140°). The curve labeled "S" shows the photon flux at absolute threshold. This is equivalent to the number of rods that receive a quantum. The fit is tolerable, and considerably better than that between the number of rods *per se* and photon flux, but the match between the slope of these functions is not better than that between retinal irradiance and rod density. This computation of the increase in "noise" in the photoreceptor sheet is thus not a very much improved predictor of psychophysical absolute threshold over the increase in rods *per se*. Perhaps whatever noise is generated in the rod network is dissipated before the 3rd order synapse. Data from retinal ganglion cells are consistent with this view, for spontaneous activity in darkness does not change with growth, even though rod input increases (Falzett *et al.*, 1988).

Possible role of higher-order cells. The first limit on threshold may be attributed to the properties of the rod photoreceptors, but the fact that photon(s) have been detected must traverse many synapses before an organism can organize an appropriate response. Where might additional limitations appear, and what insights can the data we have gathered provide?

If the surround mechanisms of individual neurons are essentially inactive at absolute threshold (Barlow *et al.*, 1957), and if these mechanisms arise through lateral interaction attributable to horizontal and (possibly) amacrine cells, then the next limiting neuron for absolute threshold must be the bipolar cell. Let us make the simplifying assumption that a bipolar cell signals a ganglion cell that its (the bipolar cell's) threshold has been reached whenever it receives an adequate signal from the rods. We assume this signal increases as some

function of N, where N is the number of rods synapsing on the bipolar cell.

Several types of bipolar cells have been identified in goldfish retina (Stell *et al.*, 1977), and the number of rods contacting the b1 type cell has been quantified for different sizes of fish over about the same range we used (Kock and Stell, 1985). Between about 3 and 19 cm sbl, rod synapses onto b1 bipolar cells increase by a factor of 1.45, due to the addition of new rods above them. These bipolar cells contact every rod within their dendritic field, and their dendritic fields are nonoverlapping.

We can use our numbers to estimate how the number of photons affecting b1 bipolar cells changes with growth. Psychophysical threshold for small fish was reached when 1 photon was incident per 1400 rods; for large fish threshold was 1 photon per 2800 rods (computed from Table 1). This is an increase in rods per photon of a factor of 2. The ratio of rods/ganglion cell increases by a factor of 5.1. If the ratio of rods/bipolar cells (perhaps of all types) is some multiple of the ratio of rods/ganglion cells, and if this factor does not change with growth, then a hypothetical threshold-detecting bipolar cell would receive $5.1/2 = 2.55$ times as many quanta at threshold in large fish than in small. These numbers fit moderately well with the idea that threshold increases as $\sqrt{N}$, because the square root of the increase in the size of the rod pool ($\sqrt{5.1} = 2.26$) approximates the increase in the number of photons seen by the hypothetical bipolar cell (2.55). Thus, the bipolar cell's signal-to-noise ratio may be another limiting factor for setting threshold.

Since psychophysical threshold is reached when 1 in 1400–2800 rods receives a quantum, we can also ask what structures have 1400–2800 rods within their receptive fields. According to Kock and Stell (1985) the number of rods per b1 bipolar is an order of magnitude lower than this, so we could guess that about 1 in 10 bipolar cells is stimulated at threshold. If a ganglion cell's receptive field is about 10× that of the bipolar (Macy and Easter, 1981; Hitchcock and Easter, 1986), this analysis suggests that a ganglion cell might report detection to the brain when 1 in 10 bipolar cells reaches threshold.

The neural determinants of absolute threshold thus certainly include (1) the length of the rod outer segments (Bassi and Powers, 1986) and (2) the planimetric density of rods in the retina (the present paper; also Powers *et al.*, 1987). More tentatively, as mechanisms of transmission of the signals arising at threshold from only a few rods, we propose (3) stimulation of a small number (estimated at 1/10, under our conditions) of bipolar cells to activate them just beyond the "noise" provided to them by the rods, followed by (4) synaptic transfer to the retinal ganglion cell viewing about 10 bipolar cells; then this cell alters its firing rate to signal "detection" to the brain.

Other possible explanations

It is possible that artifacts of the retinal stimulus and/or processing beyond the retina are responsible for the changes in threshold reported here. Entoptic scatter could have been larger in larger fish due to the larger retinal stimulus, or due to larger ocular lens size. Although we cannot rule this possibility out, it is inconsistent with recordings from retinal ganglion cells reported in the companion paper: A trend nearly identical to that observed psychophysically occurred in Off-type ganglion cells and not in On- or On/Off-type cells when their thresholds were measured under conditions like those used here (see Fig. 10 in Falzett *et al.*, 1988). An increase in sensitivity of one class of retinal ganglion cell but not another would seem to argue against an effect of stray light. An empirical way to address this question would be to repeat the psychophysical experiment with a ganzfeld stimulus (cf. Alpern *et al.*, 1987).

It is also possible that changes related to growth elsewhere in the visual system (e.g. the optic tectum) could somehow counteract the increased convergence of the rods in the retina, thus mitigating any effects of convergence on threshold. This possibility is difficult to rule out, but is also difficult to reconcile given the close parallels among rod density, Off cell sensitivity (Falzett *et al.*, 1988) and psychophysical sensitivity with growth.

Another set of explanations revolves around the issue of performance in the psychophysical task. It is possible that older fish learn or perform better or more reliably than younger fish. Our data do not support this, however, because the number of trials required during acquisition did not differ with body length, nor did the slopes of the psychometric functions that were measured from trained animals. Seasonal differences in learning (Shashoua, 1973) are also unlikely to have contributed to the results because fish were trained and tested throughout the year in both replications. Finally, higher

brain functions involved in attention or alertness could be responsible for increased sensitivity in older fish. This factor cannot be assessed at present.

Visual function during growth

The results reported here show that the process of adding new neurons to retina and brain does not interfere with the goldfish's ability to detect light at absolute threshold. More importantly they demonstrate that new retinal neurons must form functional synapses with older, existing neurons (Kock and Stell, 1982, 1985); otherwise absolute threshold could not increase with age.

New rods are added in larger numbers than other retinal neurons to the central retina as well as the periphery (Johns and Fernald, 1981; Johns, 1982; Raymond, 1985; Raymond and Rivlin, 1986). This study shows that the preferential addition of rods does increase visual sensitivity, but the increase is so small that it is probably of more interest to us as visual neurolobiologists than it is to the goldfish. For the fish, the important role of continued rod addition is to fill in spaces in the photoreceptor sheet so that rod density does not drop as the retina stretches. The functional outcome is that a given visual stimulus remains approximately equally detectable throughout life.

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RESPONSIVITY AND ABSOLUTE SENSITIVITY OF RETINAL GANGLION CELLS IN GOLDFISH OF DIFFERENT SIZES, WHEN MEASURED UNDER "PSYCHOPHYSICAL" CONDITIONS

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Abstract—Retinal neurogenesis occurs in adult goldfish, and more rods are added to the retina than any other class of cell as the fish grows. To determine whether the disproportionate addition of rods affects the responsivity and sensitivity of dark adapted retinal ganglion cells, we recorded activity from optic tract fibers in goldfish of different sizes. Experimental conditions were as similar as possible to those used in a separate study in which psychophysical absolute thresholds were measured: large, dim, monochromatic spots 1 sec in duration were projected close to the right eye of alert, self-respiring goldfish. A total of 214 fibers were recorded in small (5.0–5.7 cm), medium (9.5–11.0 cm) and large (13.0–20.0 cm) fish. Neither maintained activity (mean and variance of the discharge rate in darkness) nor responsivity (quantum-to-spike ratios) nor absolute threshold (quantal irradiance required to produce a difference of 1 spike/trial from spontaneous rates) varied reliably with size of fish. However, some Off cells were more active in the dark than On and On/Off cells; these had low QSR's and absolute thresholds, and were found in all sizes of fish. Fifty percent (50%) of Off cells (compared to 8% of On cells) had thresholds comparable to or lower than psychophysical threshold, and Off cell thresholds (but not On cell thresholds) tended to be lower in larger fish. Because psychophysical threshold is closely related to the planimetric density of rods in goldfish, the similarity between Off cell threshold and psychophysical threshold suggests that Off cells may be influenced relatively more than On cells by the addition of new rods to the retina.

Retinal ganglion cells Scotopic sensitivity Neural development Rods Goldfish

INTRODUCTION

In embryonic and larval goldfish, mitotically active cells appear throughout the retina (Sharma and Ungar, 1980; Johns, 1982). At later stages of development the neurogenesis of most cell types becomes restricted to an annular zone at the retinal margin. A notable exception to this rule is the rods: new rods continue to be added across the entire retina during adulthood, interspersed among older, already differentiated neurons (Johns and Fernald, 1981; Johns, 1982; Raymond 1985). The newly differentiated rods are known to form synapses with existing *b*1 bipolar cells, which in turn increase in somatic and dendritic field size (Stell and Kock, 1982; Kock and Stell, 1985). The possibility exists that the new rods form synapses with other types of bipolar cell as well. New synapses also continue to form within the inner plexiform layer of goldfish retina during growth (Fisher and Easter, 1979; Marotte, 1980), and ganglion cells from larger eyes have longer dendrites and wider dendritic fields than those from smaller eyes (Kock and Reuter, 1978b; Hitchcock and Easter, 1986).

Despite these many retinal changes, the absolute visual threshold of the goldfish changes very little with growth (Powers *et al.*, 1988). In this paper we ask whether the spontaneous activity, responsitivity or absolute threshold of goldfish retinal ganglion cells changes with growth. To facilitate comparison with the psychophysical measurements, we used stimulus conditions typical of our psychophysical studies and we recorded from ganglion cells in awake, self-respiring goldfish.

METHODS

Animal preparation

Procedures adhered to the ARVO resolution on the use of animals in research. Seven small (5.0–5.75 cm standard body length, tip of nose to base of tail), 7 medium (9.5–11.0 cm) and 8 large (13.0–20.0 cm) common goldfish

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(*Carassius auratus*), purchased from a commercial supplier (Ozark Fisheries Stoutland, Mo.), were maintained in the laboratory at 20°C (±1°C) on a 12 hr:12 hr light:dark cycle for at least 2 weeks before undergoing surgery. These size categories correspond to ages of < 1 yr (small), 2–3 yr (medium) and 4–5 yr (large) (Johns and Easter, 1977), and were chosen to be similar to those used in previous anatomical (Johns and Easter, 1977; Easter *et al.*, 1981; Johns, 1982) and psychophysical (Powers *et al.*, 1988) studies.

Goldfish were placed in a light-proof chamber for at least 1 hour prior to surgery, which was performed under dim red illumination in an otherwise dark room. The surgical procedure was modified from Shefner and Levine (1976). Fish were anesthetized by immersion in 0.1% tricaine methanesulfonate (Finquel, Ayerst Laboratories) until respiratory activity ceased (about 5–10 min). Under deep anesthesia the spinal cord was transected at the level of the third vertebra, leaving the innervation of gills and viscera intact. This prevented any voluntary sketetal activity, while allowing the fish to self-respire. It also eliminated sensory input from the body. The cranium was opened while the fish was still anesthetized, bilaterally exposing the caudal telencephalon and the rostral optic tectum. Fatty tissue overlying the brain was aspirated, and a local anesthetic (2% Lidocaine ointment) was applied to the cut edges of the skull.

Following surgery, which required 10–15 min, the fish was placed in a Plexiglas aquarium inside a light-proof recording cage (see Fig. 1). The animal's head was immobilized by means of a small clamp attached to the rostral edge of the skull opening, and its body was supported with sponges. Water from the fish's home tank was aerated, filtered and continuously recirculated through the aquarium. The eyes were fully immersed in water, so the corneas remained clear and optically inactive. For large fish, where respiratory movements resulted in movements of the brain, we routinely filled the cranium with an agar solution. Respiration rate was monitored routinely throughout these experiments to ensure that the animal remained healthy, and in a few cases heart rate was also monitored by means of a silver wire electrode inserted into the thoracic cavity. When either of these measures, which are known to correlate with detection of noxious stimuli by goldfish (Otis *et al.*, 1957) indicated the animals were unduly uncomfortable the experiment was terminated. It was in fact impossible to record from distressed animals due to excessive movements of the head, brain and eyes. At the conclusion of the experiment fish were sacrificed by anesthetic overdose, then decapitated and/or pithed. Body length was measured with a centimeter rule, and lens diameter was measured with calipers.

Extensive precautions were taken to ensure that the animals remained thoroughly dark-adapted throughout the experiments. Control measurements on several fish showed that the tricaine anesthesia used during surgery had no effect on psychophysical absolute threshold (Falzett, 1984).

Stimulus conditions

Figure 1 shows the optical system. Light from a regulated tungsten–halogen source (Ealing model 227–1403) was focused on a shutter (Uniblitz model 325B), collimated, and passed through a 520 nm interference filter (Melles Griot, 8 nm bandwidth at half height) and neutral density (Oriel) filters before being brought to focus again at the entrance of a 3/8″ fiber optic light pipe (Edmund Scientific). This wavelength was chosen because it is near the peak of the goldfish rod porphyropsin absorption spectrum (Schwanzara, 1967) and because previous psychophysical measurements had shown that vision is mediated by rods in this region of the spectrum (Powers and Easter, 1978). The other end of the light pipe was mounted in an *X-Y* manipulator 10 cm from one wall of the Plexiglas aquarium. White bond paper secured to the aquarium provided a rear projection screen. The light pipe produced a diffuse circular spot on the screen, and the fish's right eye was placed so that the spot subtended a visual angle of 96° regardless of the size of the fish. The spot was centered on the eye by placing an infrared filter (Kodak Wratten 89C) at *IF* and adjusting the *X-Y* manipulator while viewing the eye with an infrared image converter (FWS Systems).

Although all neurons reported here within the central 60° of retina, no attempt was made to center the stimulus on the cell's receptive field in this experiment because we were interested in the nature of the responses that might be given by retinal ganglion cells during psychophysical measurements of absolute threshold. In the companion psychophysical study (Powers *et al.*, 1988) threshold was measured with large, diffuse

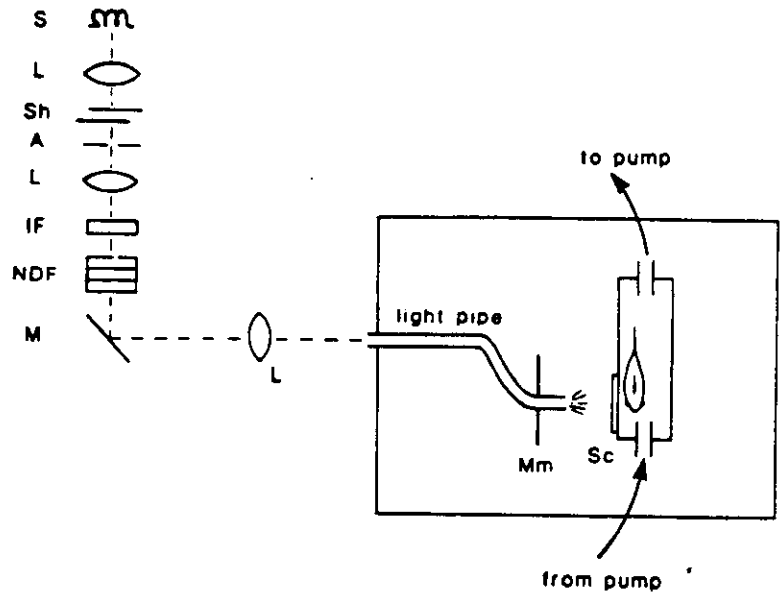

Fig. 1. Apparatus. The Plexiglas aquarium containing the immobilized fish was positioned in a lightproof recording cage, and stimuli were delivered via a light pipe from the optical system outside. Fresh water was continually circulated in the aquarium by means of a pump. S: source; L: lens; Sh: shutter; A: aperture; IF: interference filter; NDF: neutral density filter; M: mirror; Mm: micromanipulator; Sc: rear projection screen.

stimuli presented to animals that were bodily restrained but free to move their eyes. We assume that these conditions involve stimulation of many cells because of the large overlap of ganglion cell receptive fields in goldfish (Macy and Easter, 1981). We further assume that any given cell is likely to receive relatively constant stimulation as long as it is reasonably centrally located in the retina.

The quantal irradiance of the stimulus was computed from measurements made with a calibrated photodiode (PIN-10DFP, United Detector Technology) placed at the plane of the pupil. Calibrations were performed several times throughout the course of the experiment; measured values did not vary more than ± 0.06 log unit. Stimulus intensity is expressed in units of corneal irradiance (photons sec^{-1} cm^{-2} incident at the cornea) or retinal flux (photons sec^{-1} incident at the retina). Retinal flux was computed by taking into account the diameter of the fish's pupil (Falzett, 1984) and the absorption of the eye media (Bassi *et al.*, 1984). QSR's took into account in addition the area of the stimulus on the retina (Powers and Easter, 1978).

Recording techniques

Tungsten wire-in-glass electrodes (Levick, 1972) were used to record action potentials from optic tract fibers. Tip diameters of 1–3 μm with exposures of 12–18 μm provided the best isolation of individual axons. The electrode was held in a micromanipulator and positioned above the left optic tract using coordinates obtained from Peter and Gill (1975) and corrected for differences due to the fish's orientation in our apparatus. The electrode was lowered into the tract via a hydraulic microdrive until light-driven responses occurred during presentation of dim, 520 nm stimuli 1 sec in duration. The location of the electrode in the tract was verified in a histological experiment wherein current was passed across the microelectrode following recording of single units. Lesions were subsequently easily visible within the optic tract in cresyl-violet stained 40 μm frozen sections, and adjacent sections clearly showed evidence of the electrode track.

Action potentials were filtered and amplified (Differential Preamplifier, Rockefeller University), displayed on an oscilloscope (Tektronix) and monitored over a loud speaker (Haer Audio Monitor). The time base and trigger level of the oscilloscope were adjusted to generate a TTL-compatible 5V gate-out pulse with each spike; this was in turn fed into a Schmitt trigger on an LSI 11/23 computer (Data Translation). The time of occurrence of each spike was stored with 1 msec resolution on floppy disk for later analysis.

Procedure

After a fiber had been well isolated, it was classified as On, Off, or On/Off (Hartline, 1938) based on its response to a near-threshold

520 nm light. Under dark adapted conditions, On cells increase their firing rates and Off cells generally decrease their firing rates in response to near-threshold stimuli. On/Off cells increase their firing rates both at onset and offset of light, and some Off cells increase firing at stimulus offset.

Following classification, the preparation was dark-adapted for at least 30 min, and then an intensity–response series was obtained for each cell, as follows. A 520 nm stimulus, 1 sec in duration, was presented 30–50 times at an intensity that had elicited no discernible response during classification of the cell. On each trial, the computer recorded all spikes that occurred in a 3-sec interval, composed of 1 sec before, 1 sec during and 1 sec after the stimulus; activity was not recorded during an additional 1 sec intertrial interval. If the cell remained well isolated, the intensity was increased by about 0.3 log unit and the procedure was repeated. This continued until the cell gave a clear response on all trials. Most cells did so within 1–1.5 log units of the first intensity. Note that the total inter-stimulus interval was 3 sec. Control experiments using longer and shorter intervals showed that this time was sufficient to allow recovery from any adapting effects the dim stimuli may have had.

Data analysis

Spike trains were analyzed off line. To obtain measures of spontaneous activity we constructed distributions of baseline spike discharge during the 1 sec pre-stimulus interval for trials below threshold or at the lowest intensity used. Both pulse number distributions (number of spikes sec^{-1} per trial) and interpulse interval distributions (the time between successive spikes over all trials) were drawn, but the statistics reported here (the mean number of spikes sec^{-1} per trial and the variance or standard deviation of the number of spikes sec^{-1} over trials) were computed from pulse number distributions. A post hoc examination of the data revealed no obvious effect of the dim, sub-threshold stimuli on the shape of the pulse number of interpulse interval distributions.

Intensity–response functions were generated by a technique described in full elsewhere (Falzett *et al.*, 1985). The method involves computing a cumulative response function for each intensity tested from an averaged (over trials) peri-stimulus time histogram—an integral of the PST. An important aspect of the method is its ability to identify the beginning and end of the response, which was always substantially delayed relative to the stimulus interval under the dark adapted conditions of our experiment. This technique uses the statistical properties of the neuronal spike train itself to determine the beginning and end of the temporal response window, and thus provides a more accurate measure of threshold or responsivity than methods that analyze responses during the stimulus period only. This may be particularly relevant under scotopic conditions where response latencies tend to be long (see Fig. 5).

Once the end points of the response interval were identified, the magnitude of the response was determined by comparing the slopes of the different segments of the cumulative response function, which correspond to firing rates during pre-response, response and post-response intervals. Repeating this procedure at different stimulus intensities yielded intensity–response functions that show the mean number of spikes above or below baseline activity during the response interval of interest [R in equation (3) of Falzett *et al.*, 1985]. For dim lights where $R > 0$ these functions tend to be linear (Barlow and Levick, 1969), so their slopes are conveniently described by linear regression analysis. Regression equations were computed from a minimum of 3 intensities for all but 4 On cells and 1 Off cell, for which only 2 intensities produced $R > 0$. When light intensity is expressed as retinal flux (see Stimulus Conditions) the reciprocal of the slope of the intensity–response function is the quantum-to-spike ratio (QSR): the incremental number of photons per spike produced over the range of intensities tested. QSR is the measure of responsivity in this paper.

In order to compare the neuronal data to psychophysical thresholds we defined a cell's "absolute threshold" as the corneal irradiance that produced a mean change of 1 spike from baseline ($R = 1$). This value was computed from the least squares regression equation relating R to corneal irradiance (see Fig. 6 below and Falzett *et al.*, 1985).

RESULTS

We recorded from a total of 214 ganglion cells. Of these, 204 were demonstrably sensitive to light, and could be classified as to type based on their response to near-threshold stimuli.

Table 1. Number of neurons recorded

Body length	Off	On	On/Off	N. R.	Total
Small 5.15 ± 0.11 cm	22(13)	22(10)	8(5)	3	55
Medium 10.00 ± 0.24 cm	29(18)	20(8)	7(4)	4	60
Large 16.06 ± 0.88 cm	37(13)	49(19)	10(3)	3	99
Total	88	91	25	10	214

The number of ganglion cell axons from which records were obtained, by class of cell and size of fish. Body lengths are nose to base of tail, sbl ± 1 SEM. Number of fish = 7 for small, 7 for medium, and 8 for large. N.R. stands for "not responsive"; these cells could not be classified because they did not respond to visual stimuli. Maintained activity was recorded for all neurons. Values in parentheses indicate the number of cells for which complete intensity–response functions were obtained.

Table 1 shows the number of cells recorded by cell type and size of fish.

Maintained activity in darkness

All cells we encountered had some level of maintained activity when fully dark adapted. Figure 2 shows representative pulse number and interpulse interval distributions for an On cell, an Off cell and an On/Off cell. Both types of distributions are estimates of probability–density functions (Perkel *et al.*, 1967; Barlow and Levick, 1969). The interpulse interval distribution describes the probability that an interval of a given duration will occur between 2 successive impulses, and the pulse number distribution describes the probability that a certain number of impulses will occur within a given temporal window; in our case the window was arbitrarily defined as 1000 msec. Levine (1980) has shown that the variability of impulse occurrence in goldfish ganglion cells in the absence of stimulation is not a renewal process: that is, it is not strictly a random stochastic process, but exhibits short-term regularities. The regularities affect the shape of PST histograms. In the present study, the shapes of the pulse number and interpulse interval distributions did not vary systematically with body length or cell type, which were the independent variables of interest. We therefore did not analyze the structure of maintained activity any further. The statistical analyses that follow were

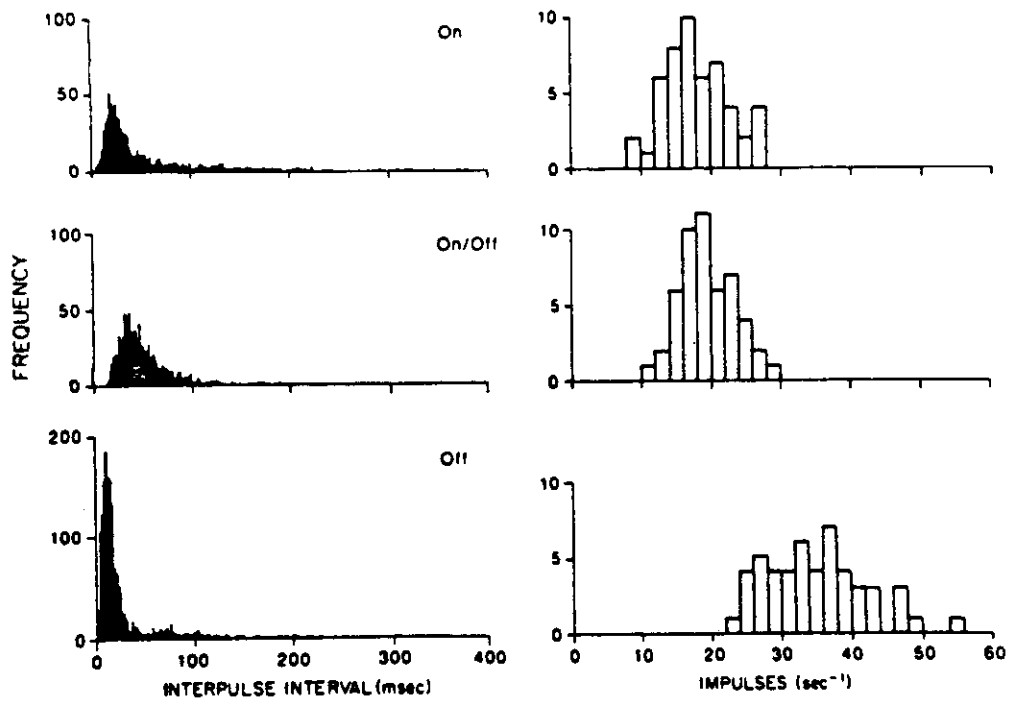

Fig. 2. Maintained activity of goldfish ganglion cells in darkness. Left panel shows interpulse interval distributions during 50 1-sec sampling periods for typical individual On, On/Off and Off cells. Right panel shows pulse number distributions for the same cells during the same 50 1-sec periods.

Table 2. Mean (± 1 SD) impulses/sec recorded from goldfish optic tract fibers in darkness

	On	Off	On/Off	All types
Small	12.10(±3.61)	22.26(±4.73)	8.75(±1.99)	15.89(±3.83)
Medium	7.28(±2.76)	19.35(±5.91)	12.09(±3.66)	14.26(±4.54)
Large	7.74(±3.11)	15.60(±5.68)	10.80(±3.83)	11.09(±4.18)
All sizes	8.71(±3.16)	18.49(±5.52)	10.51(±3.20)	13.18(±4.19)

Maintained discharge rates in darkness for all cells, by class of cell and size of fish. *N*'s are in Table 1. Analysis of variance showed no significant difference in mean discharge rate with fish size ($F = 0.90$, $P = 0.41$), although larger fish tended to have slightly lower rates. Discharge rates of different cell types were significantly different ($F = 16.44$, $P < 0.0001$): Off cells were higher than those of On or On/Off cells, which resembled each other. This was the case within each size category (i.e. the interaction beween cell type and size of fish was not significant: $F = 0.85$, $P = 0.50$). The standard deviation of the maintained discharge also did not change with size of fish ($F = 1.19$, $P = 0.31$), but did with type of cell ($F = 16.5_3$, $P < 0.0001$): Off cells were more variable than On and On/Off cells within every size category (interaction was not significant: $F = 1.23$, $P = 0.30$).

performed on both types of distributions, leading to the same conclusions. In the remainder of the paper, we discuss only pulse number distributions.

Pulse number distributions were used to compute the mean number of impulses sec^{-1} for ganglion cells in fish of different sizes. Table 2 shows that mean firing rate in the dark did not vary with size of fish, for any class of cell; the tendency toward lower discharge rates for larger fish was not statistically reliable (see Table legend). Off cells were generally more active in the dark than either On or On/Off cells, regardless of size of fish.

A similar pattern occurred with standard deviation. The values shown in Table 2 indicate the average variability of firing rate over successive trials, and again, no systematic differences were observed among the size categories. But differences did occur among classes of cell: the variability of Off cells was greater, on average, than that of On or On/Off cells.

Figure 3 shows that the variance of the maintained discharge rate correlated with the mean rate, for all classes of cell and all size categories of fish. Except for a few high-rate, high-variance cells in large fish, the scatterplots by age are similar to one another. The upper-

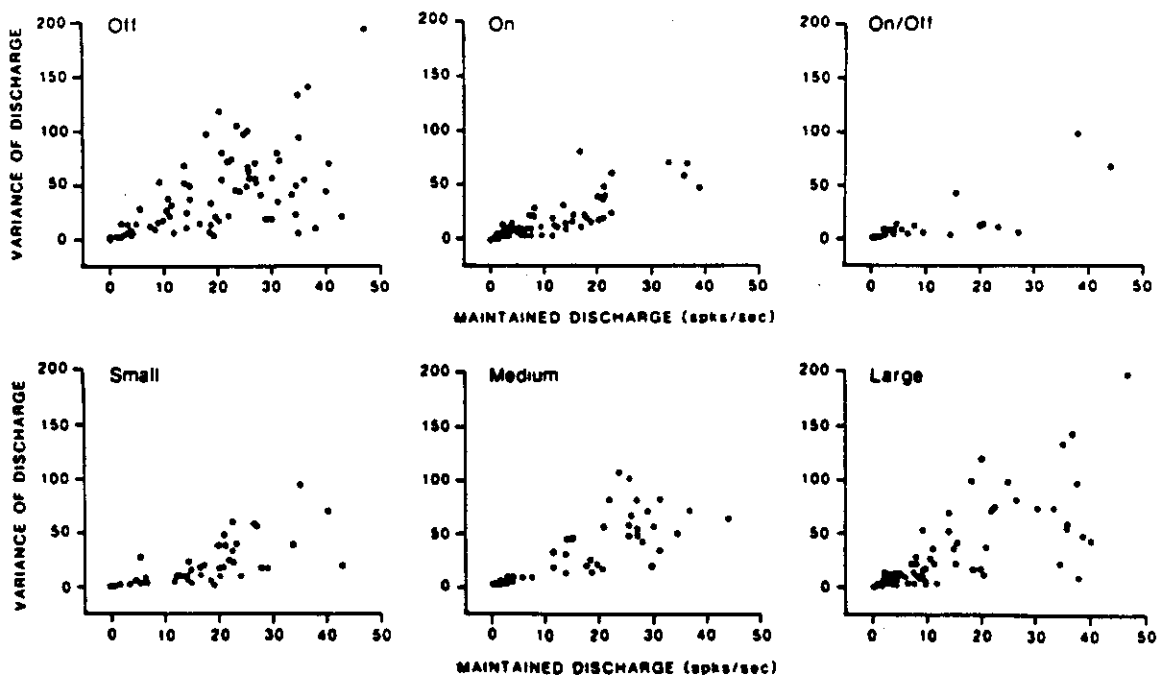

Fig. 3. The relation between mean and variance of maintained activity in darkness. Each point represents one ganglion cell, with data averaged over 50 1-sec periods. In the top graphs cells are categorized according to response type without regard to fish size; in the bottom graphs cells are categorized according to size of fish without regard to response type.

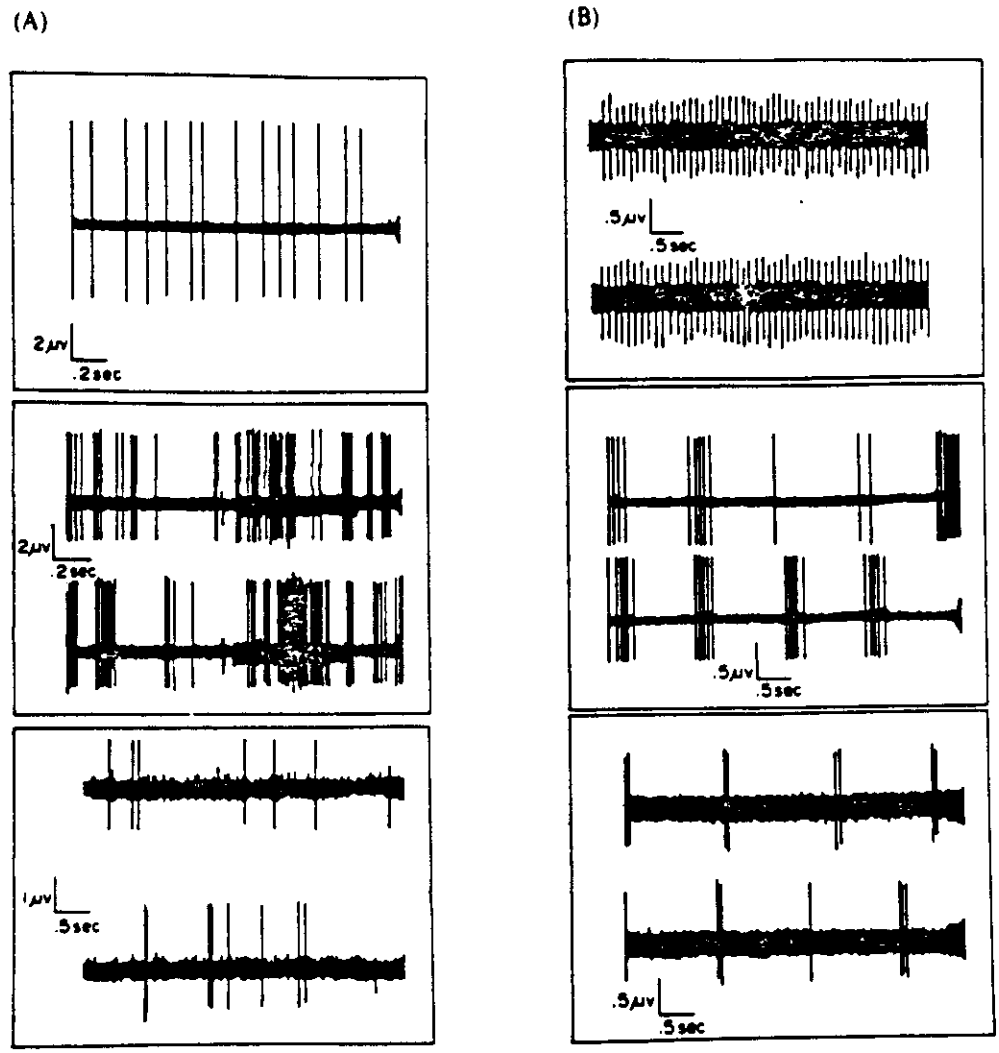

Fig. 4. Examples of patterns of maintained activity in darkness. (A) Maintained discharge recorded from ganglion cells that responded to light. (B) Recordings from 3 of the 10 ganglion cells that did not respond to light. These insensitive cells tended to have unusually regular patterns of activity in darkness.

most points in the plot labeled "Large" are in fact Off cells, as can be seen by comparing the sizewise plots with the cellwise plots above them. There were no significant differences in slope among these functions.

Unresponsive units. About 5% of the cells we encountered in the optic tract did not produce a noticeable modulation of baseline activity in response to monochromatic stimuli, even at levels that were clearly photopic to us. Broad-band white light was equally ineffective. In every case, such neurons were surrounded by other fibers, both above and below them within the optic tract, that *were* sensitive to light. And when examined closely the waveform of the action potentials always appeared normal.

Eight of the 10 unresponsive units had relatively low maintained discharge rates (< 10 spikes sec^{-1}), and all 10 had very regular firing patterns. They were evenly distributed across all sizes of fish (see Table 1). Their interpulse (or inter-burst) intervals ranged from a few milliseconds to 5 sec or more; an interpulse interval distribution for such a cell (as in Fig. 2) would be extremely narrow. One unresponsive cell fired 1-sec bursts of 150–200 spikes every 2 or 3 sec. The discharge remained regular and unaltered by visual stimuli for as long as these units were recorded, which in the best case was 40 min. Typical records demonstrating the regularity of maintained discharge in visually unresponsive units are shown in panel B of Fig. 4. Panel A shows examples of records from light-sensitive units for comparison.

Stimulated activity

Figure 5 shows examples of PST histograms from each cell type and size of fish. These histograms represent summed activity during 50

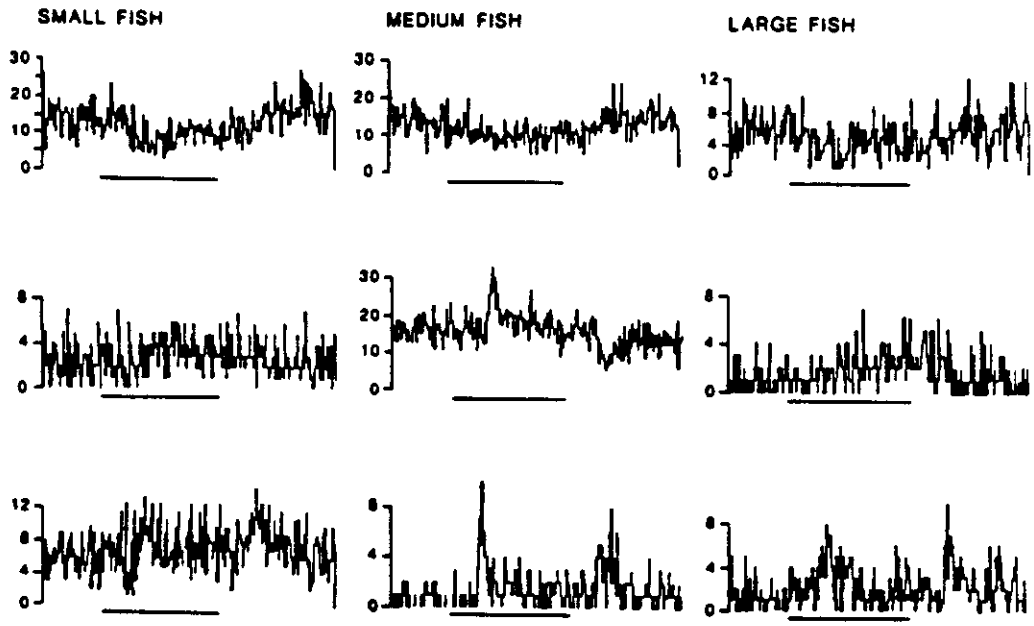

Fig. 5. Representative responses of goldfish retinal ganglion cells just above their absolute thresholds. Peri-stimulus-time (PST) histograms are shown, summed over 50 trials, for each type of cell in small (left column), medium (middle column) and large (right column) fish. The top row shows Off cells, which tended to be sustained in all sizes of fish. The middle row shows On cells, which had transient components in about 50% of the cases. The bottom row shows On/Off cells, which were transient in over 95% of cases. Ordinate: number of spikes in 50 trials; stimulus marker 1 sec.

presentations of the dimmest intensity that elicited a just-suprathreshold response ($R > 1$) under full dark adaptation. There were no obvious differences in the form of responses from different sizes of fish. On and Off cells could be either transient or sustained, and both types were found in all sizes of fish. In general more Off cells than On cells were sustained: when collapsed across fish size, 73% of Off cells were sustained, while only 50% of On cells were. With 1 exception, On/Off cells were always transient.

Figure 6 shows examples of dark-adapted intensity–response functions for each type of cell. Although the examples are from neurons recorded in the optic tracts of small fish, they illustrate our findings from cells in all sizes of fish. In small, medium and large fish, On/Off cells responded with increased spike output following both onset (circles) and offset (squares) of the stimulus. 89% of On cells in all sizes of fish increased firing after stimulus onset (circles) and returned to baseline rates after offset; the remainder increased firing following onset and decreased below baseline values after offset (not illustrated in Fig. 6). Seventy-five percent of Off cells decreased firing during stimulus presentation, returning to baseline after offset (not shown). Of the remainder, half of the Off cells responded by decreasing their firing rate during stimulus presentation (circles in Fig. 6) and increasing it after stimulus offset (squares), and half responded with increased firing only after stimulus offset. When more than one component was present in the response, QSR's and thresholds were determined by taking the mean of the values derived for each component. In the end this mattered little because the values for one component were generally close to those for the other, as Fig. 6 illustrates.

The similarity in slope of the different components of a ganglion cell's intensity–response function observed in this study may be attributable either to the conditions of the experiment or to our method of defining the response. Under dark adaptation, surround activity should be reduced or absent (Barlow *et al.*, 1957). Thus, while different aspects of the response may reflect different weightings of center and surround under photopic conditions, we would not expect to see such an effect scotopically. In terms of the method, we defined the response intervals by examining the spike train (see Data Analysis), which may give different magnitudes of response than would be obtained from setting arbitrary periods relative to stimulus presentation.

The small dot at the end of each regression line in Fig. 6 shows the best estimate of the cell's absolute threshold, expressed as the photon flux at the retina that produced $R = 1$ on average

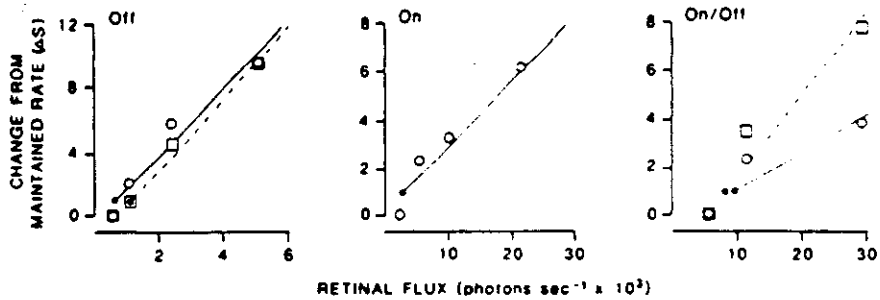

Fig. 6. Intensity–response functions for Off, On and On/Off cells (all from small fish). The points were computed from PST histograms by a cumulative sums procedure (Falzett *et al.*, 1985) which compares the rate of spike activity during an empirically-defined response interval with the rate of activity during ≥ 1 sec period preceding the response. Open circles show responses following the onset of light; squares show responses following offset of light. This On cell did not respond at light offset, unlike the On cell from a medium-sized fish shown in Fig. 5. The functions are reasonably approximated by straight lines, which have been fit by least squares regression to the data. We defined threshold, for purposes of comparison with psychophysical values, as the point on the intensity–response function where a change of 1 spike from maintained rate occurred (i.e. where $\Delta S = 1$). This point is marked by a small dot at the end of each function; when two response components were present, they rarely differed in threshold. Quantum-to-spike ratios (QSR's) were computed from the slopes of the linear regression functions, taking account of the area of the stimulus on the retina.

over 50 trials. Threshold for the Off cell in Fig. 6 was about 900 photons sec^{-1}, for the On cell it was 2500 photons sec^{-1} and for the On/Off cell it was 8700 photons sec^{-1}.

Responsivity. The total range of QSR's recorded was more than 4 log units. No systematic changes occurred with size of fish, indexed as the diameter of the ocular lens in Fig. 7 (Falzett, 1984). The arrows in Fig. 7 indicate groups of cells that were all recorded from the same fish. Under the conditions we used, responsivity varied widely from cell to cell, even within the same preparation. This variability could be due to different receptive field sizes or (less likely) different center-surround weighting, but it does suggest that fish of every size have ganglion cells that are highly responsive to large field stimuli (e.g. there were 22 Off cells and 3 On cells with QSR's < 3500) and rather unresponsive to large field stimuli (there were cells of all types with QSR's of 100,000 or more).

Although no changes in responsivity occurred with age, the distribution of QSR's did differ significantly between Off cells and On and On/Off cells (see Figure legend). On average, Off cells' QSR's were 0.47 log unit lower than those of On and On/Off cells. More striking is the nearly total lack of On or On/Off cells with log QSR's below 3.5 (about 7000 photons per spike), compared with the even distribution of Off cells below that value. Only 6% of On cells had QSR's below 7000, compared to 40% of Off cells.

How does responsivity relate to spontaneous activity? Figure 8 shows QSR's for a group of cells as a function of the standard deviation of their spontaneous activity in darkness. For this analysis we used only the best cells in the sample: those for which the intensity–response function had ≥ 4 points and an r value for linear

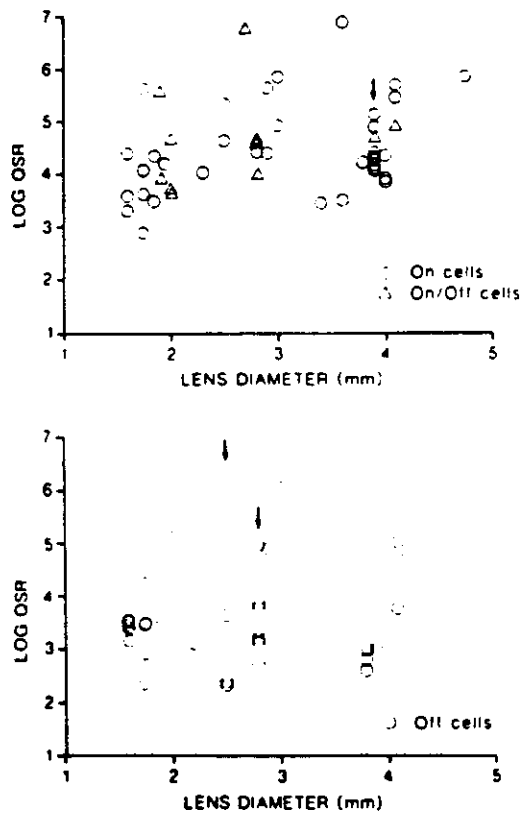

Fig. 7. Quantum-to-spike ratios (QSR's) for each cell, as a function of size of fish. The diameter of the ocular lens has been used as an index of eye size (Falzett, 1984), which correlates more closely with retinal parameters than does sbl. On and On/Off cells are plotted above, Off cells below; no changes in QSR occurred with increasing lens diameter. Arrows indicate groups of cells recorded from the same fish. Overall, Off cells had significantly lower QSR's than On cells and On/Off cells ($t = 2.900$, d.f. = 89, $P < 0.005$).

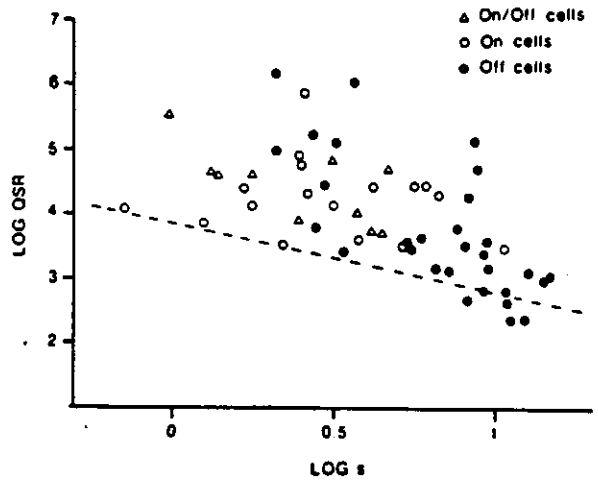

Fig. 8. Relation between log QSR and the logarithm of the standard deviation of the maintained discharge in darkness (s). Not all cells in the sample appear in this figure (see text for details). Regardless of size of fish, Off cells (solid circles) tended to be more variable and to have lower QSR's than On or On/Off cells (open symbols). The dashed line, with a slope of -1, represents a constant ratio of "signal" (QSR) to "noise" (s) on this log-log plot. A few Off cells had exceptionally low signal-to-noise ratios, by this definition, because they lie below the line that includes all other cells.

regression of at least 0.9. The standard deviation of the spontaneous activity may be taken as an indicator of "noise," and we have already shown that Off cells tend to be noisier by this definition (see Table 2 and Fig. 3). If QSR is considered to be the "signal" produced by dim lights, each point can be taken to respresent a given cell's signal-to-noise ratio.

The distributions of On, On/Off and Off cells are highly scattered, indicating that within each cell type there are individual neurons with widely varying signal-to-noise ratios. But the distributions are not identical. The Off cells are clustered at the low QSR, high s corner, while the On and On/Off cells tend to have higher QSR's and lower s. A line with a slope of -1 on this log–log plot represents a constant relaionship between QSR and "noise," or a constant signal-to-noise ratio. Such a line has been drawn into Fig. 8 at an arbitrary ratio that excludes all On and On/Off cells. The On and On/Off cells closest to the line are the most sensitive of their type because they have the highest signal-to-noise ratios. The small cluster of Off cells below the line have higher signal-to-noise ratio than any On or On/Off cells, and by this definition these Off cells ($N = 4$, or 13% of the cells illustrated in Fig. 8) were the most sensitive in the sample.

Corneal irradiance at absolute threshold. Quantum-to-spike ratios are not easy to relate to psychophysical threshold. For that reason we turn next to a measure that emphasizes the stimulus parameters in visual space, at the level of the cornea, before photons enter the eye. Figure 9 shows histograms of log corneal irradiance needed to produce a change of 1 spike, on average, in ganglion cells of all types from small, medium and large fish. The arrows show mean absolute visual threshold for fish of comparable body lengths when tested psychophysically (Powers *et al.*, 1988). The range of psychophysical thresholds was ± 0.75 log unit for all 3 size categories (Powers *et al.*, 1988). In contrast, ganglion cell thresholds spanned 3–4 log units.

Twenty-five percent (7 out of 28) of the cells we recorded in small fish had corneal thresholds that were at or below psychophysical threshold; 35% (11/29) of those from medium fish and 27% (10/33) of those from large fish had thresholds at or below psychophysical values as well. All units that had thresholds below 4.0 log photons sec^{-1} cm^{-2} were Off type.

Table 3 lists threshold values by cell type and size of fish. As with QSR's, within every size category Off cells had lower thresholds than both On cells and On/Off cells. Moreover, when averaged over fish size, Off cell thresholds were 0.51–0.61 log unit lower than the other two classes.

Threshold corneal irradiance did not change significantly with size of fish for On, Off or On/Off cells. However, Off cells tended to be more sensitive in larger fish, and the average thresholds of Off cells paralleled the change in

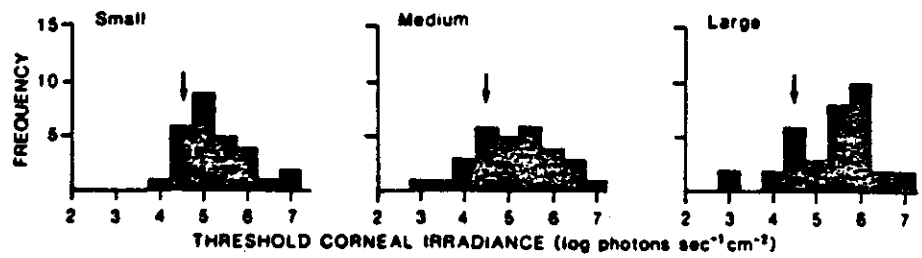

Fig. 9. Absolute threshold for all retinal ganglion cells studied in these experiments, plotted according to size of fish. Cells of all response types are combined within each size category. Arrows indicate average psychophysical threshold for fish of comparable sizes (Powers *et al.*, 1988).

Table 3. Log corneal irradiance at absolute threshold for goldfish retinal ganglion cells (photons sec^{-1} cm^{-2})

Size	On	Off	On/Off	All types
Small	5.38 ± 0.14	5.12 ± 0.24	5.73 ± 0.31	5.32 ± 0.14
Medium	5.47 ± 0.20	4.95 ± 0.27	5.15 ± 0.23	5.11 ± 0.17
Large	5.55 ± 0.17	4.84 ± 0.32	5.94 ± 0.31	5.34 ± 0.16
All sizes	5.49	4.98	5.59	

Corneal irradiance at absolute threshold for ganglion cells in dark adapted golfish. Mean photon density sec^{-1} is expressed logarithmically, ± 1 SEM, for each class of cell and size category of fish. Threshold did not vary significantly with size of fish ($F = 0.042$, $P > 0.05$), but differences were observed with cell type ($F = 3.69$, $P < 0.01$): Off cells had lower thresholds in each size of fish.

psychophysical absolute threshold (Fig. 10). This correlation suggests that activity in Off cells may be particularly relevant for determining psychophysical threshold for large diffuse scotopic stimuli at any age.

DISCUSSION

The purpose of this experiment was to determine whether the activity of retinal ganglion cells in goldfish changes as new neurons are added to the retina. We chose stimulus conditions like those used psychophysically and recorded from ganglion cell axons in awake animals in order to facilitate comparison between neuronal and behavioral measures of threshold. The results will be discussed from three point of view: their implications for the impact of retinal neurogenesis on visual function near absolute threshold, their relation to psychophysical measurements of absolute threshold in goldfish of different sizes, and the differences between responses from Off cells and On or On/Off cells near absolute threshold.

Ganglion cell activity and growth

All ganglion cells we encountered had some level of maintained discharge in darkness. Rates of discharge were highly variable from cell to cell, as in the cat (Kuffler *et al.*, 1957), but the range of variability was similar in small, medium and large fish and the statistics of the discharge did not change with growth. If the maintained discharge is the noise against which a signal must be detected (Kuffler *et al.*, 1957), this result implies that cells of very low and very high noisiness exist in every size of fish, and that the average level of noise in ganglion cells remains constant with growth even though the neural composition of the retina is continually changing.

As the goldfish grows the planimetric density of the rods increases slightly and the ratio of rods to ganglion cells increases greatly (Johns and Easter, 1977; Powers *et al.*, 1988). If quantum-like events in the rods are responsible for the production of maintained activity in ganglion cells, both of these factors would lead

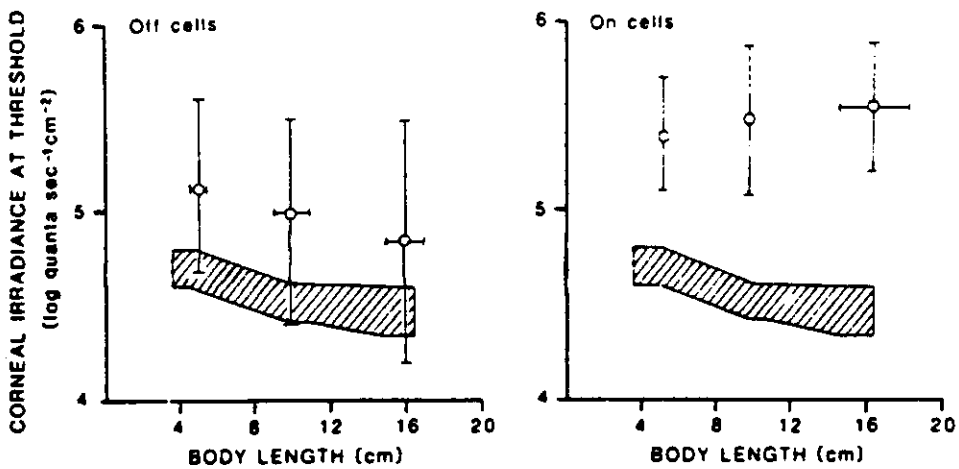

Fig. 10. Threshold for Off cells tended to decrease in parallel with psychophysical threshold (Powers *et al.*, 1988), while that of On cells did not. The points are mean log corneal irradiance at threshold for ganglion cells in small, medium and large fish. Error bars show ± 1 SEM in threshold (vertically) and in body length (horizontally). The shaded region indicates the 95% confidence region for psychophysical absolute threshold at 532 nm, computed from Powers *et al.* (1988).

to the expectation that maintained discharge rates should increase with growth. We did not observe such a change. There are several factors that could account for this result, among which are that the amount of change expected was too small to be detectable given the sample sizes we used, and that the effect of increased quantum-like events in rods was dissipated before reaching the ganglion cell level. Whatever the reason, any effects of increased noise due to increased input from rods during growth were not apparent in this experiment.

The site(s) of origin of the maintained discharge in retinal ganglion cells is unclear. Although Schellart and Spekreijse (1973) and Levine (1982) suggest that noise enters at the level of the ganglion cell itself, action potentials do not appear to arise spontaneously *within* ganglion cells, for when isolated from synaptic input ganglion cells have no maintained discharge (Rodieck, 1967; Levine, 1984). It seems more likely that the maintained discharge results from activity in cells presynaptic to the ganglion cell because the patterns of discharge in cells of like sign (On or Off) tend to be correlated (Arnett, 1978; Mastronarde, 1983; but see Schellart and Spekreijse, 1973). In correlated pairs, approximately 20% of the variability in discharge rates is due to noise source(s) that are common to both cells (Mastronarde, 1983; Ginsburg *et al.*, 1984). But the specific site of noise injection and the structures responsible for noise are still in question. Mastronarde (1983) suggested that the source of maintained activity in darkness might be quantum events mediated by cone bipolars. This would seem to implicate the receptors as originators. Johnsen and Levine (1983) propose a model for goldfish retina that is not inconsistent with this suggestion; they place the site of origin at the OPL or even distal to it, before the sign-inverting process occurs. Based on this work and our psychophysical findings (Powers *et al.*, 1988), we propose that "noise" relevant to psychophysical detection exists at all levels of retinal processing and that the exact sources responsible for such noise remain to be determined at each level.

With the possible exception of Off cells, which tended to have lower absolute thresholds in larger fish, the responsivity and absolute sensitivity of the retinal ganglion cells studied here did not change with size of fish. Thus, like the maintained discharge, these aspects of ganglion cell function do not reflect the dramatic increase in rod input suggested by the neuroanatomy (Johns and Easter, 1977; Johns and Fernald, 1981; Johns, 1982), at least when the stimuli are large, long, diffuse flashes. Whether changes would be apparent with stimuli that are better matched to the dimensions of dark adapted receptive fields remains to be determined. If receptive field sizes increase with growth, as anatomical (Hitchcock and Easter, 1986) and physiological (Macy and Easter, 1981) changes suggest, stimulation with spots that fit the centers should show that larger cells are more sensitive (Enroth-Cugell and Shapley, 1973). Such measurements remain to be made in the dark adapted goldfish.

Psychophysical measurements in goldfish have also shown minimal change in absolute sensitivity with growth (Powers *et al.*, 1988) and taken together the two studies show that having a higher ratio of rods to ganglion cells in the retina does not in itself confer higher visual sensitivity either to the ganglion cells or to the goldfish. Instead, the continued addition of rods appears to maintain the probability of photon catch approximately constant by inserting new rods to fill the spaces that would otherwise result from stretching of the retina during growth (Johns and Fernald, 1981).

Relation to pychophysical threshold

The corneal irradiance required to produce an average change of 1 spike sec^{-1} can be compared to the corneal irradiance required for visual detection. Figure 11 shows the distribution of ganglion cell thresholds superimposed on the distribution of psychophysical thresholds obtained from 29 fish of different sizes (Powers *et al.*, 1988).

Comparing psychophysical and neurophysiological measures of threshold is difficult, because of the necessarily different definitions of "threshold" involved. Part of the problem is alleviated by our use of similar stimulus conditions in the two studies; we can at least compare measurements from the same organism taken under similar conditions. But it is important to keep in mind 2 caveats during the discussion that follows. (1) Stimulus conditions were not identical. In the psychophysical experiments the stimulus subtended 140 degrees and its duration was 5 sec. In the physiological experiments reported here the stimulus subtended 96 degrees and its duration was 1 sec. If spatial and/or temporal integration continue for

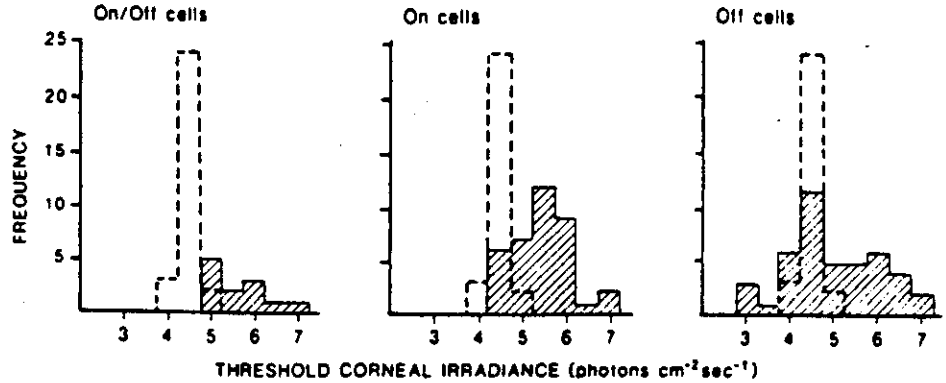

Fig. 11. Ganglion cell thresholds compared to psychophysical threshold in all sizes of fish. The dashed distribution is the same in all 3 panels. It summarizes absolute thresholds for a 532 nm, 140 deg stimulus 5 sec in duration obtained from 10 small, 10 medium and 9 large goldfish that had been classically conditioned to respond to dim lights (Powers *et al.*, 1988). In that study large fish were slightly more sensitive than smaller fish; the tails of the dashed distribution are made up of large fish to the left and small fish to the right. The shaded distributions show ganglion cell thresholds under similar conditions (520 nm. 96 deg stimulus 1 sec in duration) from the present study. The Off cell distribution extends farther into the low intensity region than the On and On/Off distributions, and is more nearly centered on the psychophysical distribution.

large, long duration targets, these stimulus differences could account for part of the difference in threshold between the sets of experiments. [Preliminary data from our laboratory suggest that the critical duration for temporal integration is < 1 sec for ERG's, ganglion cells and psychophysics (Nussdorf, unpublished observations).] (2) Two different definitions of "threshold" are involved. Psychophysical threshold is the corneal irradiance at which the conditioned inhibition of respiration reached a criterion value (half of the animal's pre-stimulus baseline respiration rate) on 50% of the trials during which the stimulus was presented (see Powers *et al.*, 1988). Threshold for retinal ganglion cells is defined in this paper as the corneal irradiance required to produce a change of 1 spike per trial from pre-stimulus firing rates, and was computed from post stimulus time histograms that had been averaged over 50 trials (see Falzett *et al.*, 1985). We do not know whether the goldfish requires this kind of input from its ganglion cells to decide whether it has seen something; our definition is based on statistical principles that may not be used by the animal in the psychophysical task. The placement of the distributions in Fig. 11 is therefore a bit arbitrary. If we had selected 60% response in the psychophysical study, the distributions outlined with dashes would move to the right relative to the ganglion cell distributions. Similar shifts would occur if different response critera had been applied to the ganglion cell data. These caveats notwithstanding, we now compare the two measures as we took them.

Note first that the distribution of ganglion cell thresholds is broader than that of psychophysical threshold (Fig. 11). Off cells are more widely dispersed than On or On/Off cells, however, and the increased dispersion is exclusively in the direction of lower thresholds: the least sensitive Off cells required 10^7 photons sec^{-1} cm^{-2} and so did the least sensitive On or On/Off cells, but the most sensitive Off cells had thresholds around 10^3 photons sec^{-1} cm^{-2}, while the most sensitive On cells required 32 times more than this before they fired an extra spike. The same general point is illustrated also in Fig. 7, where QSR's are plotted instead of thresholds.

The mode of the Off cell distribution is centered on the mode of the psychophysical distribution, although the mean threshold for Off cells was 0.5 log unit higher than the average psychophysical threshold. The mode of the On cell distribution is a full log unit higher than that of the psychophysical distribution. More importantly, only 6 of the 47 On cells (13%) responded reliably at intensities that were at or below mean psychophysical threshold. In contrast, 20 of the 43 Off cells (47%) responded at those intensities. *Thus, at corneal irradiances that were sufficient to elicit behavioral responses with a probability of 0.5, most On cells did not respond at all.* Nearly half of the Off cells, on the other hand, changed their firing rate by 1 spike per trial (on average) at such intensities. This result does not rule out the possibility that On cells could mediate detection at psychophysical levels, of course; we may have missed the more sensitive cells, or these high-threshold cells

could contribute to behavioral threshold by a means such as probability summation. But our results do suggest that Off cells would present the brain with a much larger report of the stimulus than On cells at absolute visual threshold.

This comparison further suggests that Off cells may be responsible for signaling the presence of large diffuse stimuli at absolute threshold. Even if the placement of the psychophysical and neurophysiological distributions in Fig. 11 is not completely accurate, the most sensitive cells were without exception off cells: *All* units at or below 4.0 log photons $sec^{-1} cm^{-2}$ were Off cells, and 77% of units at or below 4.5 (the average psychophysical threshold) were Off cells. Reference to Fig. 9 further suggests that Off cells may mediate absolute threshold throughout life in this species, for ganglion cells with thresholds at or below psychophysical values were distributed evenly across the 3 size categories, and most of these cells were Off type.

Difference between On and Off cells

This study has revealed some interesting differences in the activity of On and Off retinal ganglion cells in the goldfish, regardless of body length. These differences seem to be due to a subgroup of Off cells whose physiological properties differ in several ways from either On or On/Off cells when tested with large-field stimuli. Even though the purpose of this experiment was not to document these differences, we summarize them there because they were so striking, and in hopes of stimulating further research.

(1) Some Off cells were more active and more variable in darkness than any On or On/Off cell.

(2) About half of Off cells had lower QSR's than On or On/Off cells.

(3) 73% of Off cells gave sustained responses near absolute threshold, compared to 50% of On cells and 9% of On/Off cells.

(4) 27% of Off cells had lower signal-to-noise ratios than 94% of On cells (Fig. 8). Moreover, 77% (17/22) of Off cells with low QSR's had thresholds $\leqslant$ psychophysical threshold. Only 1 On cell had both a low QSR and a threshold $\leqslant$ psychophysical values. Fifteen of these Off cells were sustained-type Off cells with high maintained discharge rates, high variability *and* thresholds $\leqslant$ psychophysical values.

(5) Off cell thresholds tended to change with growth in about the same way as psychophysical absolute threshold (Fig. 10). On and On/Off cells did not follow this pattern.

Ganglion cell sensitivities varied widely, and it is likely that the stimulus conditions used in this experiment contributed to the variability. If receptive fields are not all the same size, and if surrounds remain active near threshold, then a large diffuse stimulus would not be optimal for all cells and some cells would appear to be less sensitive than they would be with more appropriate stimuli. Similarly, if the temporal summation properties of all cells are not the same some would have been better stimulated by our 1 sec spot than others. Moreover, peripherally located cells would not be optimally stimulated by a centrally located spot, and if the animal moved its eyes (which it was free to do), even centrally located cells might not receive the same retinal stimulus trial by trial. All these factors should tend to produce higher thresholds for any class of cell. To account for the differences observed here between On and Off cells, such factors would have to operate differently on different cell classes. This seems unlikely.

A factor that could have contributed to the differences between On and Off cells is electrode bias (Rodieck, 1966). If there exists a highly sensitive class of On cells with very small axon diameter, we might have missed it.

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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 μm 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 cor-

rected 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.[25]

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 μm 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 diameter (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 condition under which it was raised (cyclic, LD; constant light, LL; constant dark, DD) is indicated in the second column. The age of the animal at sacrifice (in months) and the diameter of its lens (in mm) are given. The last three animals (0NC1, 0NC3, 0NC4) were purchased as juveniles, and their ages are unknown (see text).

The lengths of cone and rod outer segments were measured in five fish from Series 2 with exposure times of 11 or 20 months. Care was taken to ensure that only intact outer segments, contained completely within the 3 μm thickness of the section, were selected for measurement.[23] Bleached sections were used for measurements because photoreceptor outer segments in unbleached sections were partially obscured by overlying melanin granules.[23] Thirty or more outer segments of three morphological types (rods, long double or single cones and short single cones) were selected from each retina and were drawn with a camera lucida at a final magnification of

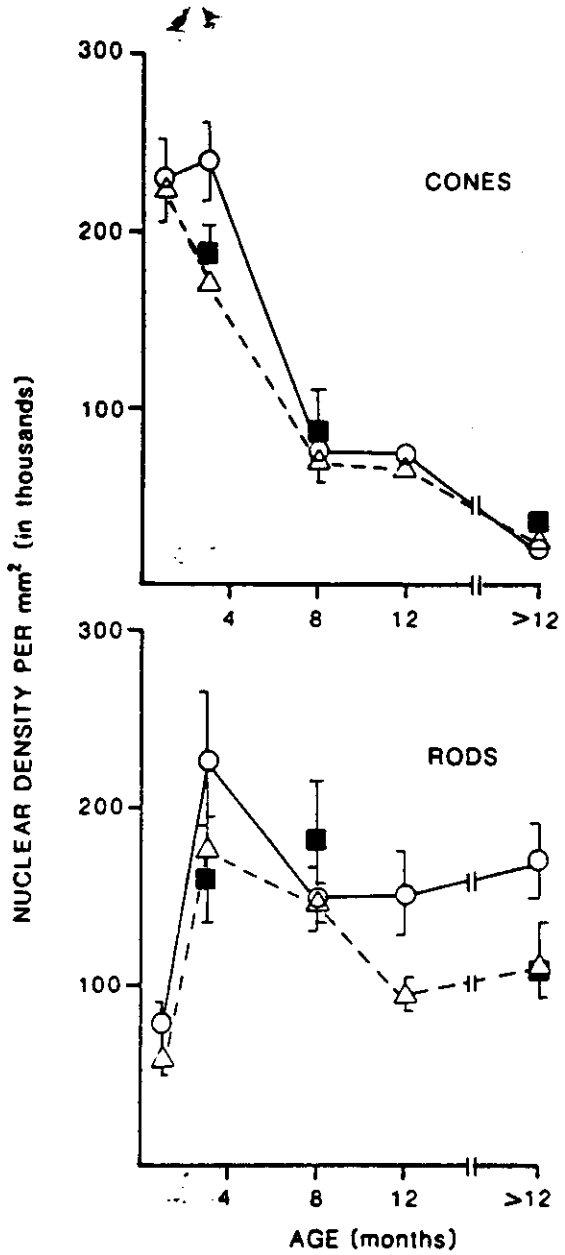

Fig. 1. Photoreceptor densities as a function of age. Cones are not affected by rearing in LL or DD: but rod density is reduced significantly after 12 months. Circles, LD; triangles, LL; filled squares, DD. Each point represents the mean of two to six retinas, with the exception of the DD value at >12 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 be-

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]; $P < 0.001$, χ^2 goodness-of-fit test). Cone densities did not differ with experimental condition. Statistical tests were not attempted with DD data.

cause 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.

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,

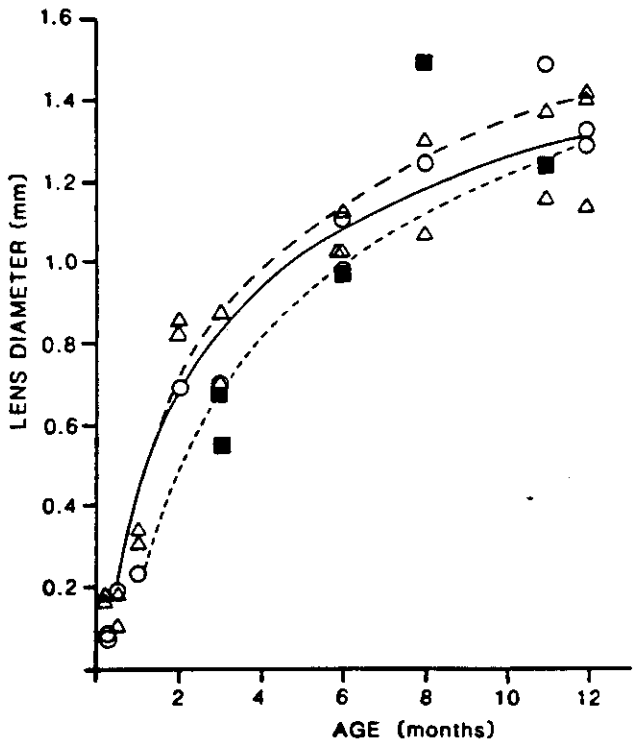

Fig. 2. Growth of fish in different lighting conditions. This graph shows lens diameter as a function of age for fish up to 12 months old, from Table 1. Each point represents an individual fish. An extra point has been added for a DD fish at 11 months; this fish does not appear in Table 1. Circles, LD; 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.

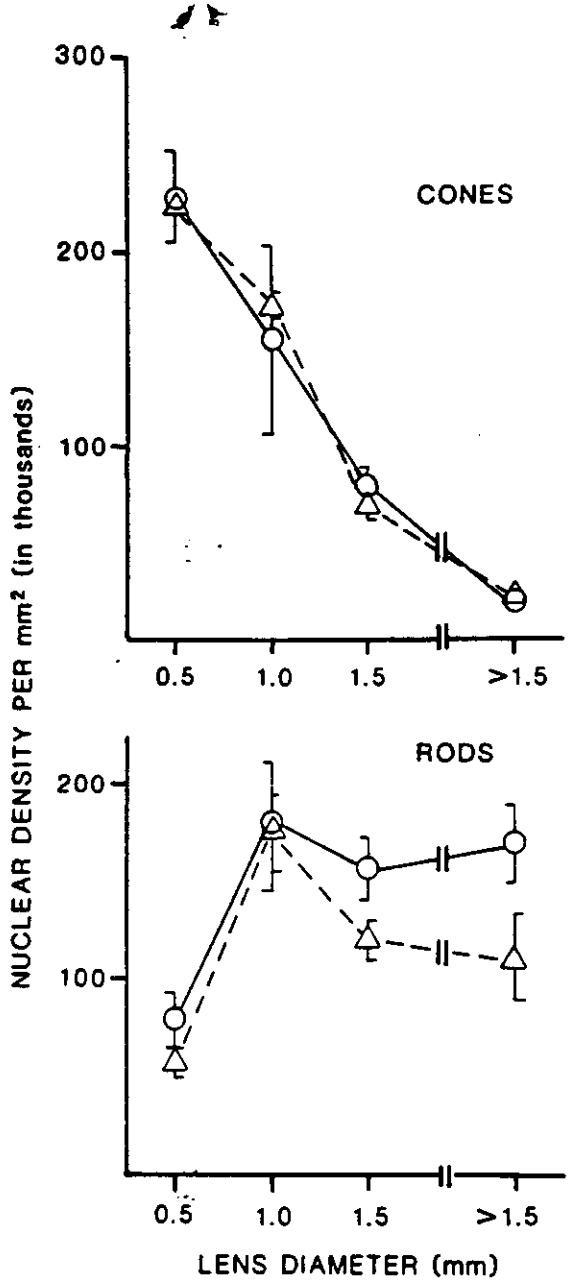

Fig. 3. Photoreceptor densities as a function of size. When differences in growth rate are factored out, the effect of rearing in LL on rods (but not cones) is still apparent. Symbols and conventions as in Figure 1. DD data omitted for clarity. Values listed in Table 3.

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)*	*SEM*
≤0.5	LD	3	228	23	79	13
	LL	6	224	4	58	8
0.51–1.0	LD	4	156	49	179	33
	LL	4	172	6	175	20
	DD	3	162	27	177	23
1.1–1.5	LD	5	79	9	157	16
	LL	10	68	6	120	11
	DD	2	49	13	129	20
>1.5	LD	3	20	2	170	21
	LL	2	22	1	111	20

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 mm^2 (in thousands) and the standard error of the mean (SEM) are given. Again, a rank sum test[40] of the difference between LL and LD fish shows that overall there were fewer rods in the LL condition ($P < 0.025$) and a χ^2 test shows that the distribution of rod densities with age is different between LL and LD fish ($P < 0.001$); LL 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 center of the retina, accounting for 5% of the total area, contains neurons that were postmitotic at hatching. At hatching the outer nuclear layer contains only cones[19] and therefore virtually all of the rods are generated postembryonically. A period of vigorous proliferation by special rod precursor cells during the first few months after hatching leads to a rapid accumulation of rods,[19,22] and our results show that this process is independent of lighting environment. Although we have not quantified retinal neurons other than photoreceptors, the normal appearance of the cellular layers in retinas from LL and DD fish, combined with the lack of an effect on number of cones, suggests that postembryonic neurogenesis of all reti-

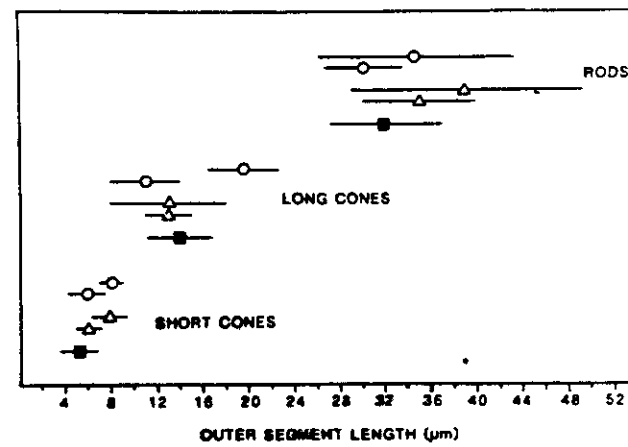

Fig. 4. Length of photoreceptor outer segments in different lighting conditions. No consistent effect of rearing in LL or DD was observed on either cone or rod OS length after 11–20 months. Symbols as in other figures. Each point is the mean of 30 or more measurements of individual photoreceptors from one retina; error 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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Lighting Conditions and Retinal Development in Goldfish: Absolute Visual Sensitivity

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Goldfish (*Carassius auratus*) were reared from hatching in constant light (340 lux), cyclic light (12 hr 320 lux, 12 hr dark) or constant dark. Absolute visual threshold was determined psychophysically in animals that still responded to visual stimuli after 1–3 years of exposure, by means of a classically conditioned respiration suppression technique wherein animals were presented with different intensities of large diffuse flashes of monochromatic light. Fish reared in constant light and fish reared in cyclic light responded reliably to stimuli above threshold, but fish reared in constant light were on average 0.58 log unit less sensitive at 532 nm, near the peak of the rod action spectrum. Two of the four fish reared in darkness did not respond to the stimuli, and thus could not be conditioned, and another fish reared in darkness responded only occasionally; threshold could not be measured in these three fish. The one fish reared in darkness that responded consistently enough to be conditioned was more than 5 log units less sensitive than normally reared fish on the first day of testing, and became progressively less sensitive over the next 2 days. Rearing under constant dark or constant light had no obvious effect on spectral sensitivity at absolute threshold. The effect of rearing in constant light on absolute threshold correlates with morphological changes in rod density,[1] but the effect of rearing in constant darkness does not. Invest Ophthalmol Vis Sci 29:37–43, 1988

In the preceding paper[1] we showed that exposure to constant light or constant dark from hatching to ≥12 months prevents the development of normal rod densities in goldfish retina. Cone densities were unaffected by rearing in either constant light or constant dark, as were the lengths of outer segments of both rods and cones. Thus, at least at the light microscopic level, exposure to constant visual conditions appears to influence only the rods, regardless of whether the conditions are constant light or constant dark.

In this paper we describe the effects of rearing in constant light (LL) or dark (DD) on the ability of the goldfish to detect dim lights. We find that LL and DD affect absolute sensitivity differently, despite their similar effects on rod density. Rearing in LL results in rather small deficits that are reasonably predictable from the 30–40% reduction in rod density observed in retinas exposed for more than 1 year to *either* LL or DD,[1] but rearing in DD produces much larger behavioral deficits that are not easy to relate to changes in rod density. The larger reduction in absolute sensitivity after rearing in DD could be related instead to the general disorganization and distortion of retinal tissue observed in these animals.

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Materials and Methods

Animals and Exposure Conditions

Procedures adhered to the ARVO Resolution on the Use of Animals in Research. Five goldfish (LL1, LL2, LL3, DD4 and DD5) were reared from embryos placed in continuous light (LL) or continuous dark (DD) prior to hatch. These embryos were obtained by breeding adult *Carassius auratus* purchased from Ozark Fisheries (Stoutland, MO), and were from the same spawns as fish used in Series 1 and Series 2 in the companion morphometric study.[1] Two additional fish (DD1, DD3) were placed into DD at 3 months of age. They had been purchased as embryos from Carolina Biological Supply (Burlington, NC) and were maintained in a combination of fluorescent room illumination and natural daylight before the experiment began.

Five fish purchased as juveniles from Ozark Fisheries were cyclic light controls (LD). They were approximately the same size (nose to base of tail, 4.8 ± 1.18 cm) as the experimental animals at the time of testing, and were maintained under daylight fluorescent lighting (12L:12D) throughout the experiment.

Details of light exposure were described in the previous paper.[1] Fish were kept three to five per tank in 10 gallon aquaria in three windowless rooms. The daylight fluorescent bulbs at ceiling height in LD and LL rooms provided 320 lux (86 $\mu W/cm^2$) and 340 lux (91 $\mu W/cm^2$) at the water's surface, respectively. DD fish were housed in a lightproof cabinet in a photographic darkroom. All animals were fed once a day. LD fish were always fed during the light part of the cycle; DD fish were fed with the aid of a dim red (Kodak Wratten filter 29, Rochester, NY) flashlight.

Conditioning was first attempted when the fish were large enough to fit into a modified restraining box of the type used by Powers and Easter.[2] Animals smaller than about 3 cm standard body length (sbl) could not be tested in this apparatus because their heads did not remain reliably positioned and because it was difficult to handle them without injury. In this set of experiments (the present paper and ref. 1) animals reached 3 cm sbl at 1–3 years (M. Powers, unpublished observations). We could not measure thresholds in fish younger than 1 year.

Measurement of Absolute Threshold

From previous work we know that detection threshold of 9–10 cm goldfish is reached at retinal fluxes of about 1 quantum per 2000–4000 rods, when the stimulus is a large, long duration flash near the peak of the rod absorption spectrum.[2] It has also been demonstrated that cones contribute to the psychophysical action spectrum in the fully dark-adapted goldfish, so that the spectral sensitivity of this animal is considerably broader than would be predicted from the rod action spectrum alone.[2] Because the spectral sensitivity of normally reared goldfish has been well specified previously, in a similar apparatus under nearly identical stimulus conditions, in the present study we measured threshold at two or three wavelengths and compared the results to the earlier values. Absolute threshold was first measured at 532 nm, near the peak of the rod absorption spectrum. Fish were subsequently tested at 636 nm and (for LL animals) at 452 nm.

To measure absolute threshold, fish were trained to suppress respiratory movements when they detected a suprathreshold light, and they were then tested for their responses to successively dimmer lights.[2] All stimuli were presented to well dark-adapted fish, with no background illumination present.

The apparatus and stimulus conditions have been described before.[2] Briefly, the fish was held in a restraining box suspended from the side of an aerated 10 gallon aquarium in a lightproof enclosure, with the right eye adjacent to a rear projection screen. Monochromatic stimuli were produced from a quartz-halogen source by placing narrow-band interference filters (Melles Griot, Irvine, CA, bandpass at half height 8–10 nm) in a collimated portion of the beam. Intensity was varied in approximately 0.3 log unit steps with neutral density filters (Melles Griot).

Training: Training was accomplished by means of a classical conditioning paradigm.[2] Fish were dark-adapted for at least 1 hr prior to each training session, regardless of experimental condition. They were then presented with 10 trials of a 5 sec, 532 nm diffuse spot, 140 deg in angular subtense, followed by a 5–15 V tail shock, 100 msec in duration. Animals showed no ill effects of this treatment, remaining healthy and eating well throughout the experiment when returned to their home aquaria. Onset of the shock (the unconditional stimulus) was contiguous with offset of the light (the conditional stimulus). The intertrial interval was variable, with an average of about 1.5 min. Unless otherwise noted, the intensity of the training stimulus was 3–4 log units above absolute threshold for 532 nm in normal fish.[2] Training sessions were repeated daily until the fish became conditioned (see next paragraph) or until the experimenter judged that the training was not effective.

Respiration rate was monitored with a glass bead thermistor placed near the fish's mouth.[2] During each intertrial interval six 5 sec samples of breathing rate were taken, to be compared to breathing rate during stimulation. A "response" was defined as ≥50% decrease in respiration rate from the average intertrial rate. When the fish responded to eight out of ten stimuli in two successive training sessions we considered it to be conditioned, and testing was begun.

Testing: Threshold was measured in trained fish from frequency-of-seeing curves derived from responses in a staircase psychophysical procedure.[2] The fish was dark-adapted for at least 1 hr before the first stimulus was presented; this stimulus was 2–3 log units above absolute threshold for normal fish, or an intensity to which the experimenter knew from previous sessions the fish would respond. If the fish responded, the intensity was decreased by 0.3 log unit on the next trial. This procedure continued until the fish did not respond to the visual stimulus. At that point the intensity was increased by 0.3 log unit until

a response again occurred. Shock followed each visual stimulus on every trial during testing.

Test sessions for LD fish were terminated after 25 trials. Sessions for LL and DD fish contained a variable number of trials, depending upon how consistent the animal's responses had been (see below). All stimuli were the same duration as training stimuli. Data for a given animal were combined across sessions to yield frequency-of-seeing curves; absolute threshold was defined as the intensity for which the probability of response was 0.5.[2]

For LD animals, two to three sessions of 532 nm stimuli were followed by two to three sessions of 636 nm stimuli. The procedure was somewhat different for LL and DD animals, because we wanted to obtain data as rapidly as possible. For these fish, if the experimenter observed at least five reversals in intensity within a session (ie, oscillations of the stimulus intensity around threshold), he or she could decide to test another wavelength within the same session. For all animals, the sequence of wavelengths was 532, 636, 452 nm.

Threshold values at each wavelength are reported in units of quanta per sec incident per cm^2 of cornea, as computed from calibrations made during the experiment by placing a calibrated photodiode (United Detector Technology, Culver City, CA, PIN10 DFP) at the plane of the pupil.[2] Absolute sensitivity, plotted in Figures 1 and 2, is the reciprocal of threshold corneal irradiance determined in this way.

Optomotor Responses

The results to be described below show that dark-reared fish were difficult to train in the classical conditioning task. For this reason, we adopted a second test of visual function that required no training. After ten unsuccessful classical conditioning training sessions, one fish (DD5) was light-adapted for 1 hr and placed in a 15 cm diameter clear Plexiglas cylindrical aquarium centered in a field of vertical square-wave stripes. The striped fields were photographic enlargements of Ronchi gratings. They could be rotated at different speeds clockwise or counterclockwise with respect to the axis of the cylinder. This apparatus was illuminated with ordinary fluorescent room illumination (Sylvania F40/CWRS/SS; 620 lux or 150 $\mu W/cm^2$ at tank level) throughout the test, which took about 15 min. Stripes subtending 27 deg, 13 deg, 7 deg and 2 deg^{-1} were rotated at various speeds around the fish, while an observer recorded the animal's following behavior (swimming in the same direction as the stripes and/or reversing direction when the stripes reversed). Two different observers (MKP and CJB) scored the fish's behavior independently, using the same stimulus set. For comparison, LL2 and LL3 were also tested in this apparatus, as was an LD control animal.

Results

Control Animals (LD)

Goldfish reared in cyclic light became conditioned after three to six training sessions. Their mean absolute visual threshold, in units of corneal irradiance, was 4.66 log quanta sec^{-1} cm^{-2} (±0.07 sem) at 532 nm. At this intensity, only one rod in about 3000 absorbs a photon each second. This low quantum-to-rod ratio, together with the similarity of the value to previous measurements[2] makes it likely that the rod system was mediating visual responses in LD fish at their absolute threshold for seeing.

Day-to-day variability in threshold for individual LD fish at 532 nm ranged from 0 to 0.38 log unit (see Table 1). The number of training sessions needed to acquire the detection task and the amount of variability in threshold are comparable to values reported before for larger fish reared in LD[2] but the thresholds reported here are slightly higher. This difference reflects normal developmental changes in absolute sensitivity in this species, which correlate closely with changes in the planimetric density of the rods during growth, even in adulthood.[3]

The dark-adapted spectral sensitivity of fish reared in cyclic light was also normal. The data from LD animals are shown in the upper portion of Figure 1, superimposed on a smooth curve from the earlier study, where complete spectral sensitivity functions were obtained.[2] The curve from the previous study has been placed on the vertical axis so that it passes through mean log threshold for LD fish (in this study) at 532 nm. The spectral sensitivity of the dark-adapted goldfish reflects input from more than one receptor mechanism, and normally does not match the absorption spectrum of rod porphyropsin in this species.[2] Thresholds for the LD fish are consistent with this finding, in that the relative sensitivity to 636 nm is higher than would be predicted from the absorption spectrum of the porphyropsin in goldfish rods.[4] Absolute threshold was 0.92 log unit higher at 636 nm than at 532 nm for the normally-reared fish in this experiment, compared to an expected difference of 1.40 if rods were mediating photon catch in the long wavelengths.

Effect of Rearing in Constant Illumination (LL)

Table 1 gives the number of training sessions and the day-to-day range in threshold for the three fish

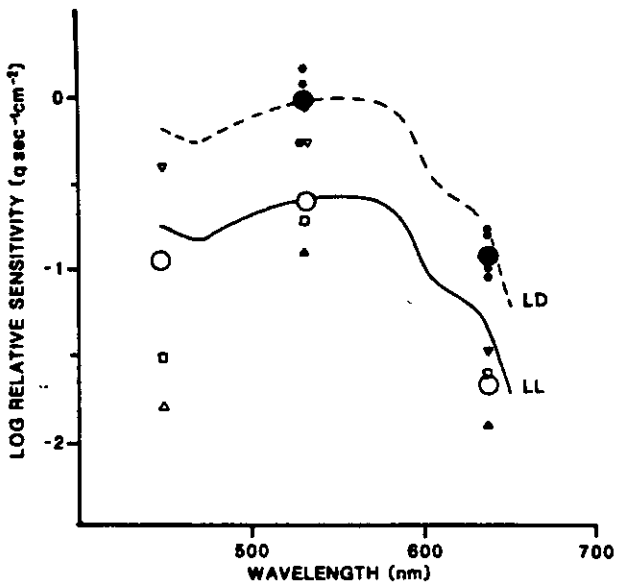

Fig. 1. Goldfish reared and maintained in continuous room illumination for 2–3 years show decreased sensitivity to large diffuse flashes of light; spectral sensitivity seems to be less affected than absolute sensitivity. Threshold of dark-adapted LL fish (open symbols) was lower than LD controls (filled symbols) at the three wavelengths tested. Large symbols show means; smaller symbols show data for individual animals. LL1 (open squares) was tested after 3 years of exposure, LL2 (open triangles) and LL3 (open inverted triangles) after 2 years (see Table 1 for details). The curve drawn through the points is the dark-adapted spectral sensitivity of adult goldfish from Powers and Easter.[2] Zero on the ordinate = 4.66 log photons sec^{-1} cm^{-2} incident at the cornea.

reared for 2–3 years in constant light. Although the Ns are small, no obvious differences were noted between animals tested after 25 months exposure to LL and the fish tested after 38 months. The number of sessions required to train all three fish was well within the range of the LD fish, and the day-to-day variability of two of the three LL fish was also within the range of normally-reared fish. We interpret this to mean that rearing in LL did not produce a generalized learning or performance deficit.

All three LL fish had higher absolute thresholds than LD fish for 532 nm stimuli (Table 1). The difference due to rearing condition was highly significant ($t = 4.919$, $df = 6$, $P < 0.005$). On average, LL fish were 0.58 log unit less sensitive than normally reared fish. This is equivalent to a 26% reduction in sensitivity.

The lower curve in Figure 1 shows the dark-adapted absolute sensitivity of light-reared animals at different regions of the spectrum. All points are plotted relative to the mean log threshold for LD fish at 532 nm, again illustrating that LL fish were less sensitive than LD fish at this wavelength. All animals were less sensitive at 636 nm, but the difference in sensitivity between 532 nm and 636 nm was slightly larger, on average, for LL fish than for LD fish (0.90, 1.03, and 1.23 log units, respectively, for LL1, LL2 and LL3, compared to an average of 0.92 log unit for LD fish). This difference in *spectral* sensitivity between LL and LD fish was not statistically significant. The data at 452 nm are somewhat more variable, but together with the long wavelength points they suggest little change in spectral sensitivity at absolute threshold in light-reared goldfish. If anything, the action spectrum is somewhat narrower in animals reared in LL, suggesting relatively less influence of cones on absolute spectral sensitivity due to rearing in LL.

Effect of Rearing in Constant Darkness (DD)

Animals reared in DD were dramatically less responsive to light than those reared for comparable times in LL (Table 2). Of the four animals tested after 12–25 months exposure, only one responded regularly enough during training sessions to be considered conditioned (see below). Two of the others did not respond at all, even to lights of different wavelength (636 nm) or high intensity (nearly ten orders of magnitude above absolute threshold for LD animals). We tried to condition one fish (DD1) twice, at 12 and 24 months of age, without success; this animal was re-

Table 1. Absolute sensitivity of light-reared fish at 532 nm

Fish	*Age at begin exposure (months)*	*Exposure time (months)*	*Number of training sessions**	*Threshold (log q sec^{-1} cm^{-2} at 532 nm)†*	*Day-to-day range in threshold‡ (log)*
LD (N = 5)	—	—	4.2 (3–6)	4.66 (4.47–4.89)	0.11 (0.00–0.38)
LL1	0	38	4	5.24	0.42
LL2	0	25	3	5.40	0.11
LL3	0	25	3	5.07	0.28

* Number of training sessions required to obtain two successive sessions wherein P (response) ≥0.8. Mean (and range) is given for LD fish. Sessions were ten trials each.

† Absolute threshold, determined as described in text, where P (detection) = 0.5. Mean (and range) is given for LD fish. LD values represent data pooled over two test sessions for four of the LD fish and data from one session for the fifth. LL values were obtained over two to three sessions.

‡ The range of threshold values obtained over the various days of testing. Mean (and range) is given for LD fish.

Table 2. Visual responses of dark-reared fish

Fish	*Age at begin exposure (months)*	*Exposure time (months)*	*Number of training sessions**	*Max percent responses/ session†*	*Successfully conditioned*
DD1	3	12	15	0	No
		24	5	0	No
DD3	3	12	13	0	No
DD4	0	25	4	100	Yes
DD5	0	25	10	20	No

* Number of training sessions required to obtain two successive sessions wherein P (response) ≥0.8 (DD4 only) or to judge that the fish was not trainable (DD1, DD3, DD5). Sessions were ten trials each.

† The percent of trials that elicited a response per session on the best day for each fish.

turned to DD for 12 more months after the first series of training sessions at 12 months.

Figure 2 shows the absolute sensitivity of DD4, the only fish we could train, at two wavelengths over 3 successive days of testing. This fish became conditioned in the usual number of training sessions (Table 2), but during the first test session its absolute threshold at 532 nm was 5.7 log units higher than the average LD fish, and 5.1 log units higher than fish reared in LL. Absolute sensitivity declined even further during the 3-day testing period shown in Figure 2, and finally, on day 4 of testing, DD4 would no longer respond to stimuli that were the maximum intensities we could produce with our optical system: 9–10 log units above absolute threshold for normal animals.

Reference to Table 1 shows that thresholds for LD and LL fish varied by a maximum of 0.42 log unit over testing days. Yet for fish DD4, sensitivity over the 3 days of testing spanned 2 log units, with sensitivity on day 2 lower than on day 1 and a further decline in sensitivity on day 3. Clearly this fish's day-to-day variation in threshold was not due to random factors, as could be argued for LD and LL fish.

Because DD4 was successfully conditioned, it seems unlikely that the animals reared in DD did not respond because they could not learn or could not organize an appropriate response. Observations with DD1 and DD3 also implied this was not the case, because they did show some evidence of respiratory suppression on the initial trials of some training sessions. Nonetheless, we tested the last DD animal (DD5) in an optomotor drum, where visually-mediated following behavior is reflexive.[5] After 25 months exposure to DD and failure to become trained in the classical conditioning task, DD5 followed stripes of 13 deg visual angle under fluorescent room illumination. This fish did not follow stripes of 2 deg subtense, and 7 deg stripes produced intermittent following behavior. In contrast, LL2 and LL3 followed stripes of all sizes, as did a control LD fish. The smallest subtense tested was 2 deg.

Condition of the Retinas in LL and DD

The retinas of four fish from this experiment were examined following completion of testing. Histological procedures were described in the previous paper,[1] where the state of these retinas was summarized under the heading "Additional comments on retinal structure." Here we describe the tissue in more detail.

The retinas of LL2 and LL3 appeared normal. There was no obvious derangement of photoreceptors or of any other structure, and no evidence of folding or scalloping. Both these fish learned the detection task, but had thresholds that were elevated above normal. Poor fixation precluded photoreceptor counts, but we presume densities would approximate those reported before.[1]

The retinas of DD4 were badly disorganized, with scalloping throughout except for a small central segment around the optic disk. This fish learned the detection task but had extremely high thresholds. The retinas of DD5, a fish that did not learn the task, were disrupted and folded across the entire extent, but not as badly as DD4. However, there was no sparing of the area surrounding the optic disk in DD5's retinas. It was not possible to determine photoreceptor densities in the DD retinas because of their overall disorganization.

Discussion

Goldfish reared in constant light or constant dark have reduced sensitivity to light at 1–3 years of age. While the effect of rearing in DD may be worse than that of rearing in LL, the magnitude of the damage due to LL is considerably smaller than that reported for mammals reared under similar conditions[6] (however see ref 7) and could be related to capacities for regeneration and continued growth in goldfish not seen in mammalian species.

The reduction in sensitivity appears to be approximately equal across the spectrum in both LL and DD animals. Although only two or three wavelengths

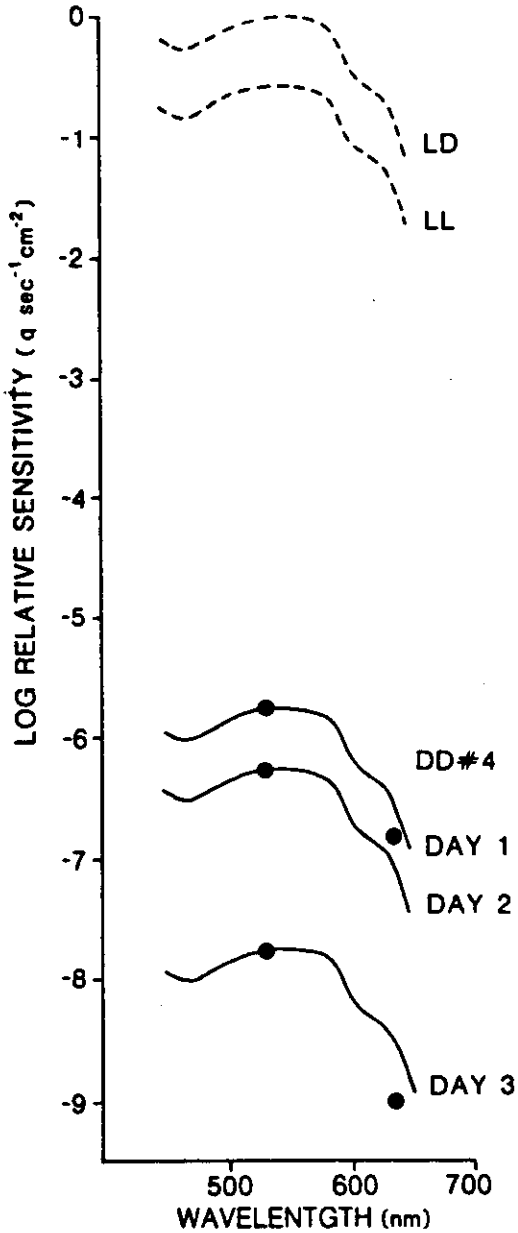

Fig. 2. Dark-adapted visual sensitivity of a goldfish reared and maintained in continuous darkness for 2 years. Ordinate same as Figure 1. The upper two curves show dark-adapted sensitivity of LD and LL fish for comparison. On the first day of testing, the fish's threshold was 5.7 log units higher than normal fish. It became progressively less sensitive on days 2 and 3 of testing, and on day 4 would no longer respond to stimuli 9–10 log units above threshold for normal fish. Day 1 and 3 curves have two points, Day 2 has one.

were used in this experiment, they were selected to take advantage of the fact that the goldfish is mesopic at absolute visual threshold.[2,8] The 532 nm stimulus was chosen to be near the peak of the rod-mediated part of the spectrum, and the 636 nm stimulus stimulates long-wavelength sensitive cones in normally reared goldfish.[2] Under this assumption, we conclude that the rod and long-wave cone *systems* were affected approximately equally by rearing in constant lighting conditions, even though only the rods were reduced in density.[1]

Goldfish reared in constant light had thresholds that were on average 26% higher than fish reared in cyclic light. In the companion study we found that fish treated identically to the LL group had reduced density of rods. The magnitude of the deficit in rod density was 37%, on average, for seven fish after about 1 year in LL.[1] The similarity between these numbers implies that the reduction in sensitivity is related to the reduction in rod density. This conclusion is consistent with the general notion that absolute visual threshold is regulated by the planimetric density of rod photoreceptors in goldfish.[3]

Attractive though this hypothesis is, alternate interpretations cannot be ruled out. It is possible, for example, that fish reared in LL suffer damages unrelated to vision that impair their ability to learn or to perform a visual task. This seems unlikely given that the number of trials required to become conditioned and the number of sessions required to obtain thresholds did not differ between LL and LD fish. A more likely explanation is that dark adaptation was incomplete in LL animals; that their ability to regenerate porphyropsin was impaired somehow by long-term exposure to light. Or, exposure to darkness following LL could have triggered massive shedding of ROS[9,10] which in turn could have interfered with the absorption of photons. Indeed, there is evidence that absolute threshold is related to ROS length in goldfish.[11] Whatever the actual mechanism, we find it remarkable that exposure to continuous light for up to 3 years has such small effects on the goldfish's ability to detect light.

Interpretation of the effect of constant darkness during rearing is more problematic. On the surface, it would appear that dark rearing produces more damage than rearing in LL, but we offer that conclusion tentatively because only one animal could be tested. One of the fish reared in DD that did not become conditioned followed the stripes in an optomotor drum, but its acuity (under photopic conditions) was at least a factor of three lower than LL and LD fish. This suggests an impairment unrelated to vision, or perhaps one that is specific to the rod system. The animal that became conditioned seemed to lose sensitivity progressively over test sessions; would this fish have shown sensitivity closer to LD fish if we could have tested it on the first day of exposure to light (instead of spending 4 days training it)? This result raises the possibility that retinas of goldfish reared in darkness are highly susceptible to damage by light, as is the case in the rat.[12]

The difference in absolute sensitivity between animals reared in LL and those reared in DD is not easily explained by changes in photoreceptor densities observed at the light microscopic level. In the companion study, we found no differences in the number of rod or cone nuclei between LL and DD fish, no differences in outer segment lengths, and no differences in the overall disorganization of the photoreceptor layer.[1] Yet when tested behaviorally, DD fish gave little evidence of vision while LL fish performed quite well. This anomaly could be related to the condition of the retinas of the particular fish used in this experiment: the dark-reared specimens were badly scalloped and folded, while the light-reared specimens were not. Another possibility is that the difference in absolute threshold between LL and DD fish is due to an effect at the ultrastructural level, perhaps related to the number or density of synaptic connections between neurons; this remains to be investigated.

Finally, we must ask whether younger fish, with shorter exposures to constant lighting conditions, would show similar deficits. We were unable to test fish younger than about 1 year in this experiment due to technical limitations; when those limitations are overcome, it will be important to determine whether shorter exposures consistently produce larger behavioral effects than can be accounted for by the retinal effects, especially in goldfish reared in constant darkness.

Key words: goldfish, absolute threshold, constant light, retinal development, constant dark

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