

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; Brancheck, 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 mm² 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

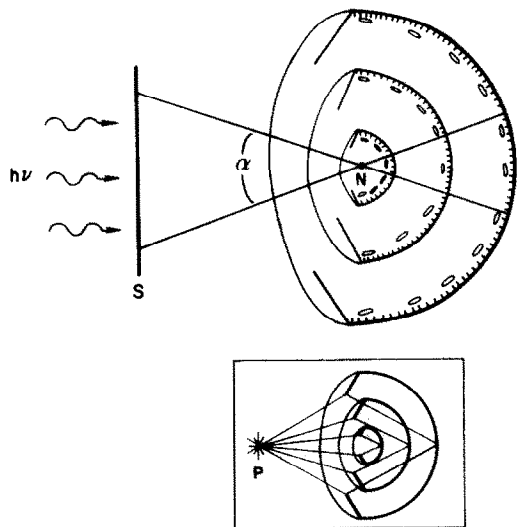


Fig. 1. Schematic drawing of goldfish retinas from three different sizes of fish (from parameters given in Easter *et al.*, 1977). Top: because of the precise scaling of the eye with growth, a visual stimulus of subtense α at plane S relative to the nodal point of the eye, N , provides photons to progressively 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

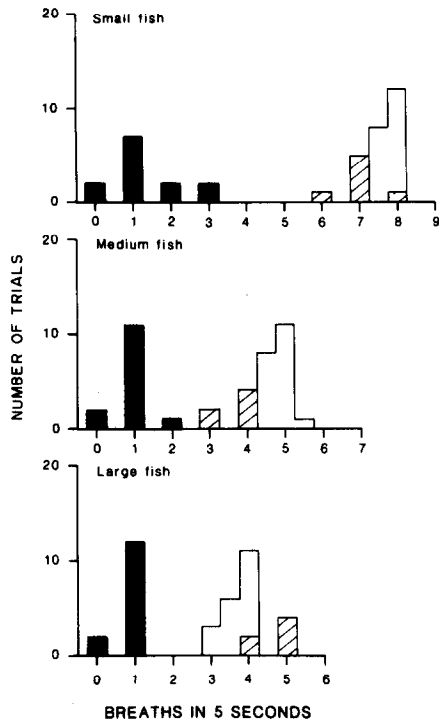


Fig. 2. Histograms of respiration rates during single test sessions for a small, a medium and a large fish. The number of breaths in 5 sec is shown for every trial, with pre-stimulus values indicated by open bars and rates during presentation of the stimulus indicated by shaded and hatched bars. The 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 $\text{cm}^{-1} \text{sec}^{-2}$ 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% glutaraldehyde, 1–3% paraformaldehyde and (in some cases) 1% picric acid. Tissues were dehydrated to 95% ethanol, embedded in Sorvall Embedding Medium (Dupont), sectioned at $3 \mu\text{m}$ 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 $100 \mu\text{m}$ 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 ($\#/\text{cm}^2$) 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

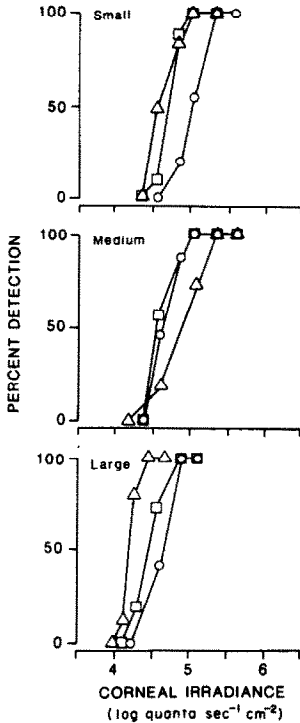


Fig. 3. Sample psychometric functions from 3 small, 3 medium and 3 large goldfish. Examples were selected to illustrate the range of sensitivities; all other functions in a given size category fell within the extremes shown here. The percent of trials on which a given fish responded is given as a function 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.

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

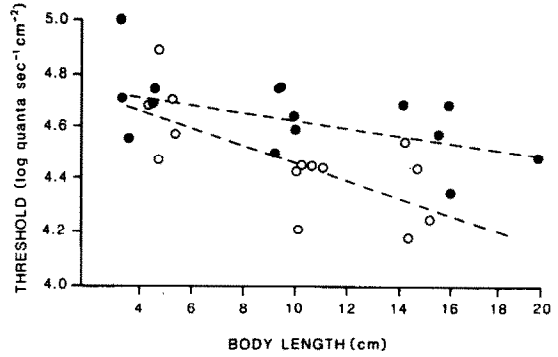


Fig. 4. Absolute visual threshold for 29 fish of different sizes. Threshold irradiance at the cornea is plotted as a function of standard body length for each subject. Open and solid points represent the results of different experiments; threshold decreased significantly with body length in both experiments by approximately the same amount. The two lines represent the least squares regression equations for each experiment ($T_c = -0.015 \text{ sbl} + 4.79$, $r = 0.54$, $P < 0.05$ for solid circles; $T_c = -0.033 \text{ sbl} + 4.79$, $r = 0.70$, $P < 0.01$ 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) \times 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 ($F_{2,23} = 1.64$, $P < 0.25$).

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

$$T_c = -0.021 \text{ sbl} + 4.77 \quad (1)$$

where T_c = absolute threshold, in log quanta $\text{sec}^{-1} \text{cm}^{-2}$ 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

	<i>N</i>	Body length in cm ($\bar{x} \pm \text{SEM}$)	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 = $2.36 \times$ 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.

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

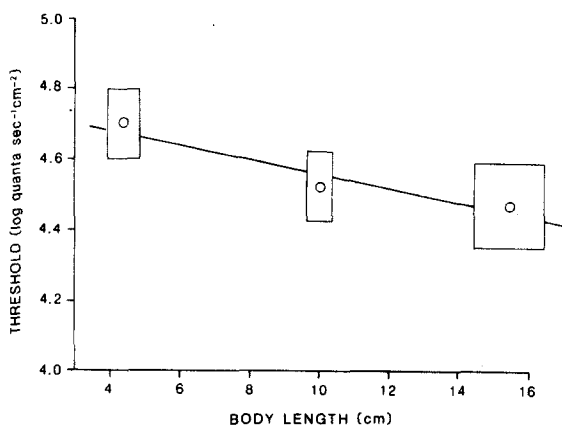


Fig. 5. Summary of the relation between absolute threshold and standard body length in the goldfish. The points are the mean log corneal irradiance for small, medium and large fish, from the data in Fig. 4. The boxes show ± 2 SEM for threshold and for body length. The solid line is the best fit by least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

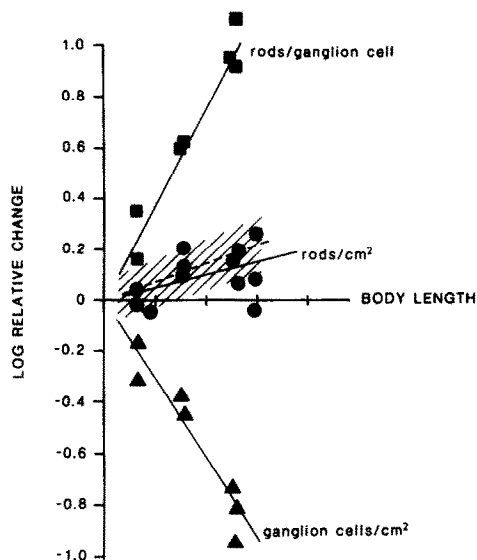


Fig. 6. Comparison of absolute sensitivity, density of rods, ganglion cells, and rod: ganglion cell ratio. See Methods for details concerning cell counts, and Table 2 for the slope of each function. To facilitate comparison, log relative increase or decrease in a given parameter is shown as a function 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: $\log \text{ rods cm}^{-2} = 0.0092 \text{ sbl} + 7.10$ ($r = 0.40$, d.f. = 11, NS); $\log \text{ ganglion cells cm}^{-2} = -0.063 \text{ sbl} + 3.89$ ($r = 0.95$, d.f. = 6, $P < 0.01$); $\log \text{ rods:ganglion cell} = 0.074 \text{ sbl} + 1.18$ ($r = 0.98$, d.f. = 5, $P < 0.01$).

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

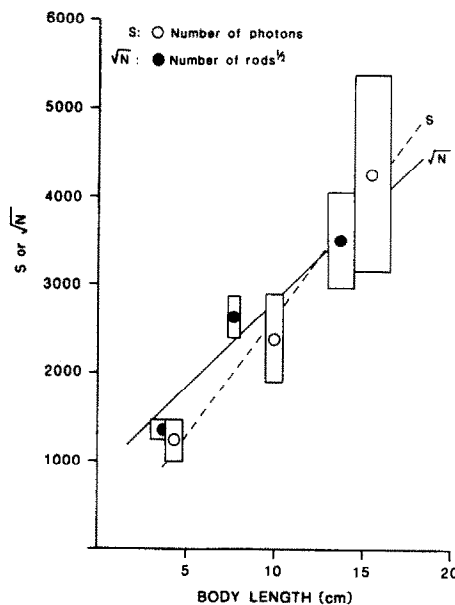


Fig. 7. Because the pupil enlarges with growth (see Fig. 1) the retinas of larger fish receive more photons at threshold. This figure shows the relation between the photon flux at threshold (open symbols) and the square root of the number of rods covered by the retinal stimulus (solid symbols).

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
$\sqrt{\text{Number of rods}}$	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 ≤ 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 ≥ 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, plani-

metric 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 sb1, 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 $10 \times$ 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 neurobiologists 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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