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"Responsibility and Absolute Sensitivity of Retinal Ganglion Cells in Goldfish of Different Sizes, When Measured Under "Psychophysical" Conditions"

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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

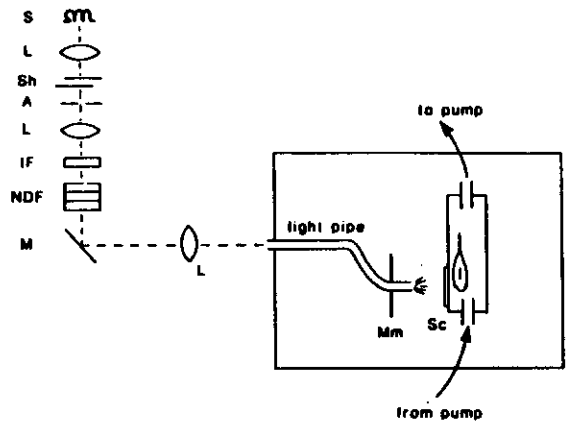

Fig. 1. Apparatus. The Plexiglas aquarium containing the immobilized fish was positioned in a lightproof recording cage, and stimuli were delivered via a light pipe from the optical system outside. Fresh water was continually circulated in the aquarium by means of a pump. S: source; L: lens; Sh: shutter; 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

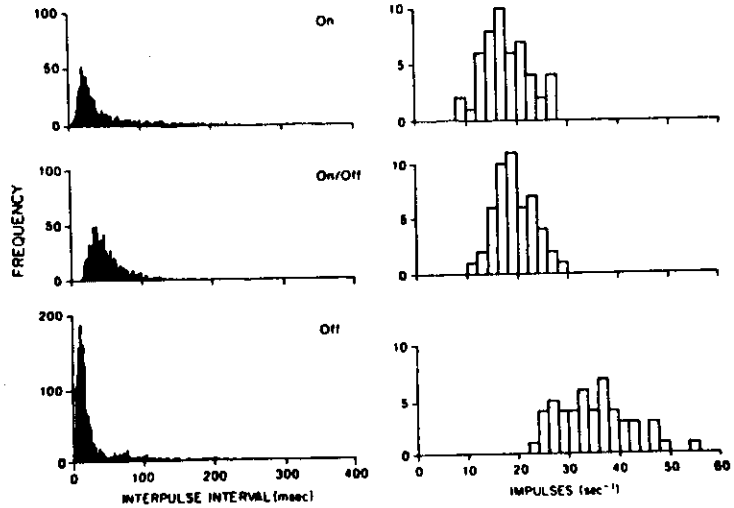

Fig. 2. Maintained activity of goldfish ganglion cells in darkness. Left panel shows interpulse interval distributions during 50 1-sec sampling periods for typical individual On, On/Off and Off cells. Right panel shows pulse number distributions for the same cells during the same 50 1-sec periods.

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 ($F = 16.5$, $P < 0.0001$): 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-

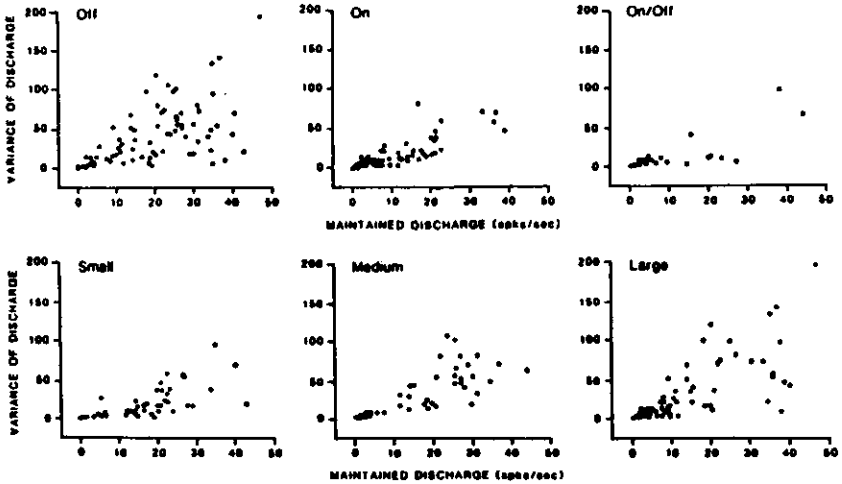

Fig. 3. The relation between mean and variance of maintained activity in darkness. Each point represents one ganglion cell, with data averaged over 50 1-sec periods. In the top graphs cells are categorized according to response type without regard to fish size; in the bottom graphs cells are categorized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

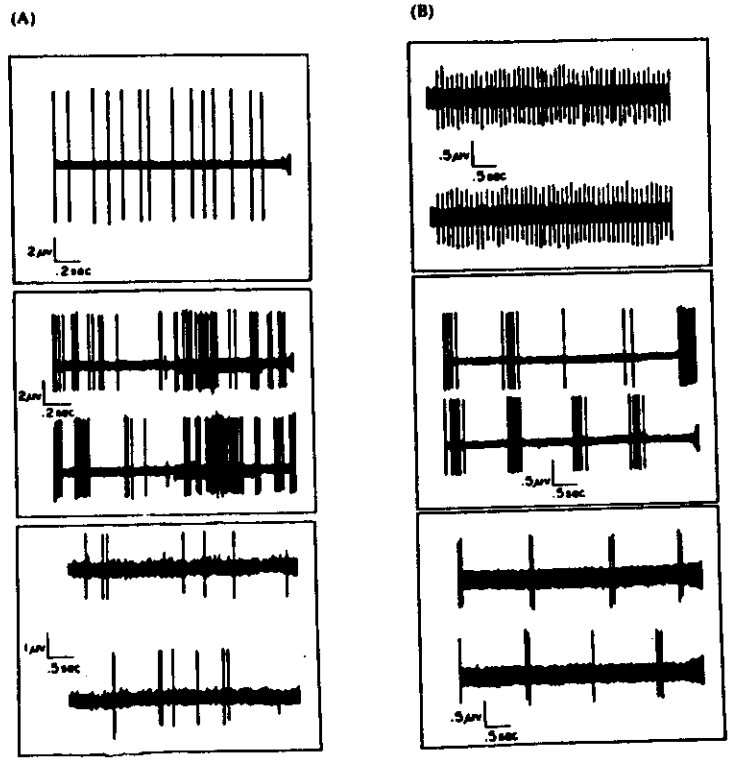

Fig. 4. Examples of patterns of maintained activity in darkness. (A) Maintained discharge recorded from ganglion cells that responded to light. (B) Recordings from 3 of the 10 ganglion cells that did not respond to light. These insensitive cells tended to have unusually regular patterns of activity in 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 inter-burst) intervals ranged from a few m seconds to 5 sec or more; an interpulse inte distribution for such a cell (as in Fig. 2) w be extremely narrow. One unresponsive fired 1-sec bursts of 150–200 spikes every 3 sec. The discharge remained regular and altered by visual stimuli for as long as t units were recorded, which in the best was 40 min. Typical records demonstratin regularity of maintained discharge in vis unresponsive units are shown in panel Fig. 4. Panel A shows examples of records light-sensitive units for comparison.

Stimulated activity

Figure 5 shows examples of PST histog from each cell type and size of fish. T histograms represent summed activity duri

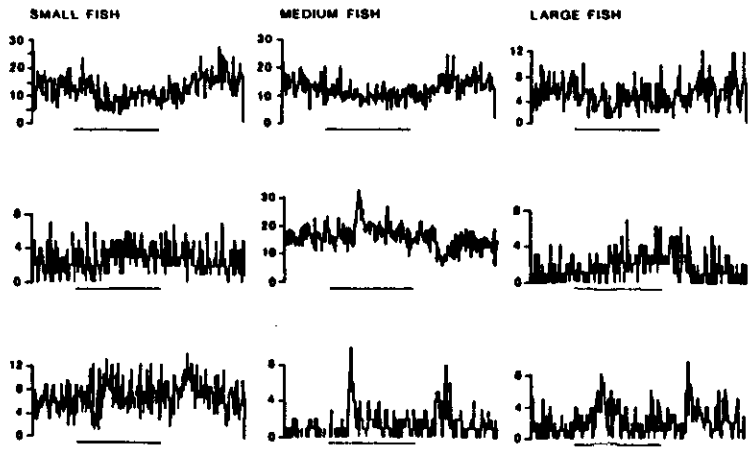

Fig. 5. Representative responses of goldfish retinal ganglion cells just above their absolute thresholds. Peri-stimulus-time (PST) histograms are shown, summed over 50 trials, for each type of cell in small (left column), medium (middle column) and large (right column) fish. The top row shows Off cells, 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.

ntations of the dimmest intensity that elic- a just-suprathreshold response ($R > 1$) un- ull dark adaptation. There were no obvious rences in the form of responses from rent sizes of fish. On and Off cells could be r transient or sustained, and both types found in all sizes of fish. In general more cells than On cells were sustained: when psed across fish size, 73% of Off cells were ined, while only 50% of On cells were. 1 exception, On/Off cells were always ient.

gure 6 shows examples of dark-adapted sity–response functions for each type of Although the examples are from neurons rded in the optic tracts of small fish, they trate our findings from cells in all sizes of In small, medium and large fish, On/Off responded with increased spike output wing both onset (circles) and offset ares) of the stimulus. 89% of On cells in all of fish increased firing after stimulus onset les) and returned to baseline rates after t; the remainder increased firing following t and decreased below baseline values after t (not illustrated in Fig. 6). Seventy-five ent of Off cells decreased firing during stim- presentation, returning to baseline after t (not shown). Of the remainder, half of the cells responded by decreasing their firing 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

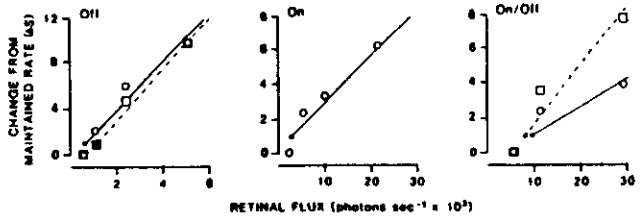

Fig. 6. Intensity–response functions for Off, On and On/Off cells (all from small fish). The points were computed from PST histograms by a cumulative sums procedure (Falzett *et al.*, 1985) which compares the rate of spike activity during an empirically-defined response interval with the rate of activity 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

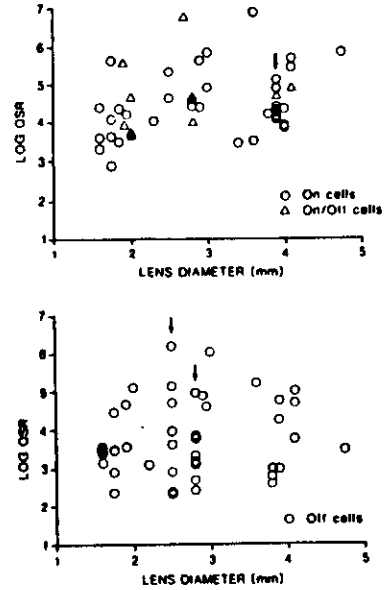

Fig. 7. Quantum-to-spike ratios (QSR's) for each cell, as a function of size of fish. The diameter of the ocular lens has been used as an index of eye size (Falzett, 1984), which correlates more closely with retinal parameters than does sbl. On and On/Off cells are plotted above, Off cells below; no 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, $P < 0.005$).

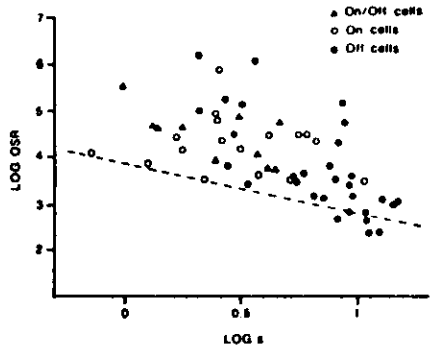

Fig. 8. Relation between log QSR and the logarithm of the standard deviation of the maintained discharge in darkness (s). Not all cells in the sample appear in this figure (see text for details). Regardless of size of fish, Off cells (solid circles) tended to be more variable and to have lower QSR's 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

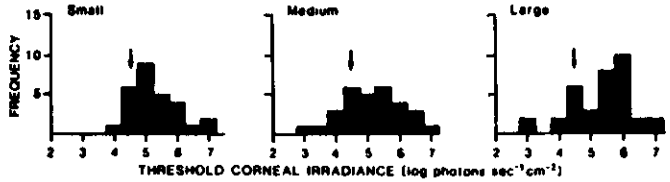

Fig. 9. Absolute threshold for all retinal ganglion cells studied in these experiments, plotted according to size of fish. Cells of all response types are combined within each size category. Arrows indicate average psychophysical threshold for fish of comparable sizes (Powers *et al.*, 1988).

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

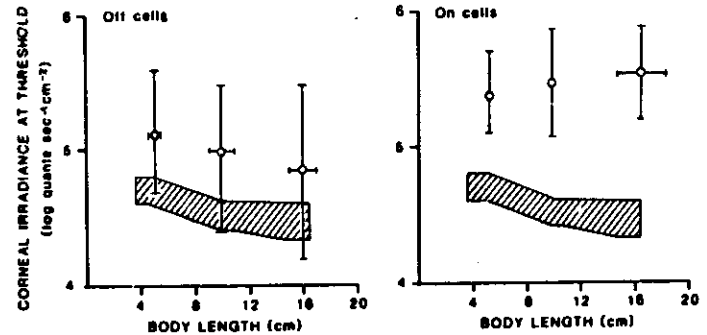

Fig. 10. Threshold for Off cells tended to decrease in parallel with psychophysical threshold (Powers *et al.*, 1988), while that of On cells did not. The points are mean log corneal irradiance at threshold for ganglion cells in small, medium and large fish. Error bars show ± 1 SEM in threshold (vertically) 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

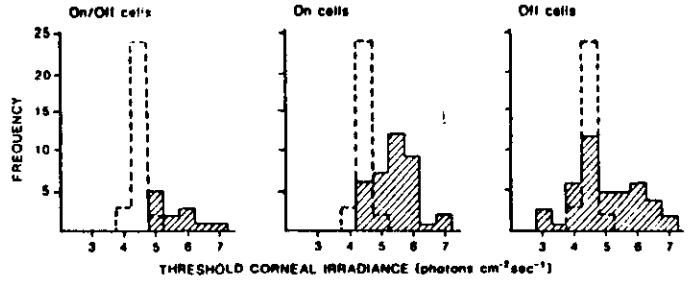

Fig. 11. Ganglion cell thresholds compared to psychophysical threshold in all sizes of fish. The dashed distribution is the same in all 3 panels. It summarizes absolute thresholds for a 532 nm, 140 deg stimulus 5 sec in duration obtained from 10 small, 10 medium and 9 large goldfish that had been classically 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 $\lesssim$ 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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