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RESPONSES TO SINGLE QUANTA OF LIGHT IN RETINAL GANGLION CELLS OF THE CAT

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MANY years ago HECHT, SHLAER and PIRENNE (1942) showed that the absorption of about 7 quanta of light in the human retina was sufficient to elicit a sensation of light. They showed, furthermore, that each absorption was likely to occur in a different receptor cell; a receptor must respond to a single quantum, and the requirement for 7 must be imposed at a higher level in the visual pathway. Since then much progress has been made in understanding the sequence of events that follows absorption. The prosthetic group, retinal, of a protein molecule, rhodopsin, is isomerized (HUBBARD and WALD, 1952) and this causes hyperpolarization of the receptor (TOMITA, KANEKO, MURAKAMI and PAUTLER, 1967) by increasing the electrical resistance of the outer segment (TOYODA, NOSAKI and TOMITA, 1969). HAGINS, PENN and YOSHIKAMI (1970) have analyzed the extracellular currents and have shown that light reduces a sustained current flow entering the rod outer segment, and this leads to the increased negative intracellular potential. Through complex synaptic structures these changes are transmitted to intermediate bipolar cells and then to the ganglion cells, whose axons reach the visual centers of the central nervous system (WERBLIN and DOWLING, 1969). It is not easy to see how the change of a single molecule can successfully initiate this sequence of events, and unfortunately the methods used for discovering the early steps are not capable of detecting the small changes evoked by weak flashes of light. The ganglion cells, however, transmit all or none action potentials, and at this level it should be possible to detect any meaningful signal that is supplied by the retina to the brain. We have, therefore, measured the absolute sensitivity of cat retinal ganglion cells in order to see how optic nerve impulses are related to quantal absorptions in the retinal receptors, using the methods of analysis described previously (BARLOW and LEVICK, 1969a, 1969b).

METHODS

We took special care both with the radiometric calibrations of light sources and the preparation of experimental animals in the present series because we hoped to resolve disagreements between earlier experiments performed in Berkeley and Canberra. The radiance of a feed-back stabilized fluorescent source

was calibrated before and after the experimental series against three standards, at three wavelengths obtained with interference filters. Two of the standards (NPL, England, and CSIRO, Australia) agreed with each other to within 1 per cent; the third (GAMMA, San Diego, U.S.A.) was 7 per cent higher, some of this error possibly being attributable to the NBS standard on which the commercial standard was based (ANONYMOUS, 1970). Calibration efforts were concentrated on the light passing the interference filter (Baird-Atomic, type B1) which was set close to the eye in all experiments. The band is 13 nm wide at 50 per cent peak transmission, and the peak is at 509 nm. The calibrations are expressed as the number of quanta of 507 nm that would have the same scotopic effectiveness. Human absolute thresholds based on these calibrations gave results within the normal range [82·5 to 146 quanta (507 nm) measured at the cornea]. The cats were prepared for retinal recording as described previously (BARLOW and LEVICK, 1969a) except that Fluothane anesthesia was used instead of Ether for induction. Contact lenses were applied, and a 3 mm dia. artificial pupil was placed as close as possible to the natural pupil which was dilated with atropine. Considerable care was taken to apply and record the optimal spherical refractive correction; use of an additional spectacle lens significantly alters the quantity of light entering the eye. In the successful preparations very few retinal penetrations by the electrode were required and the optics were clear with barely detectable corneal stippling under the protective contact lens at termination on day 2; post mortem examination revealed healthy lungs and clear trachea and bronchii. However, we also had several less successful preparations showing various degrees of pulmonary infection; these preparations were definitely less sensitive.

All units were in the retinal region backed by the tapetum and lay within about 5° of the area centralis. Pulse number distributions and post-stimulus time histograms were accumulated simultaneously on a digital computer (Hewlett–Packard 2114A) interfaced to the experiment. The computer was also used to generate impulse interval histograms of various types.

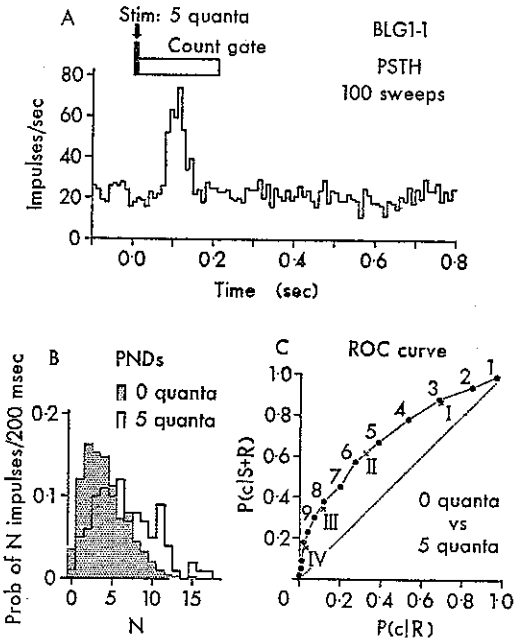

FIG. 1. Responses of a single retinal ganglion cell to 5 quanta (average) of light (507 nm) delivered to the cornea in 10 msec flashes. (A) Post-stimulus time histogram; bin width 10 msec, 100 repetitions. Count gate: 200-msec period during which the impulses are counted on each trial to generate the pulse number distribution shown in (B). Solid outline: distribution for stimulus present; dotted area, stimulus absent. (C) "Receiver-operating-characteristic" obtained from the data in (B); for number of impulses, c, as parameter, the curve plots the probability $P(c/S + R)$ that c or more impulses will occur in the presence of stimulus plus random "dark light" against the probability $P(c/R)$ that c or more impulses will occur from the dark light alone. Arabic numerals and dots show the points corresponding to various values of c. Roman numerals and crosses indicate the expected points for an ideal detector if 18 per cent of the quanta at the cornea were effectively utilized in the presence of a background noise source equivalent to 6·5 quanta at the cornea per counting period

(see p. 93).

RESULTS

Figure 1 shows the response of a single ganglion cell to a stimulus, of duration 10 msec and dia. 16 min, which delivered to the cornea the scotopic equivalent of 5 quanta (average) of 507 nm light. The average number of impulses during the 200 msec counting period, determined from 100 repetitions, was 6·62 whereas a similar counting period (400 repetitions) with no stimulus contained 4·14 (average) with standard deviation 2·67. By the convention previously adopted (BARLOW and LEVICK, 1969a), the "threshold" of this ganglion cell was 15·5 quanta at the cornea; such a stimulus could have been detected in 50 per cent of trials by observing the impulses in this single optic nerve fiber, and the detection criterion adopted would allow only a small (about 0·2 per cent) proportion of false positive responses.

TABLE 1. SUMMARY OF EXPERIMENTAL RESULTS SIMILAR TO THOSE OF FIGS. 1, 2 AND 3 FROM DARK ADAPTED ON-CENTER UNITS

Unit reference	Dark light, R_e	Threshold (cornea)	Attenuation, Q_eSR	Multiplication, m	Estimates of quantum efficiency		
					$\frac{1}{Q_eSR \times m}$	Poisson sum	Probit analysis
BLG:1	6·5	15·5	2·02	3·01	0·165	0·166	0·117
BLF:1(1)	12·3	25·7	3·94	2·22	0·114	0·097	0·081
BLF:1(2)	13·1	37·7	5·59	2·40	0·074	0·068	0·074
BLD:1(1)	3·2	16·8	2·78	2·64	0·136	0·101	0·118
BLD:1(2)	3·6	12·4	2·23	2·46	0·183	0·129	0·207
BLD:3(1)	8·8	21·4	4·30	2·09	0·111	0·119	0·115
BLD:3(3)	16·5	39·2	5·06	2·02	0·098	0·091	0·100
BLC:1(1)	23·5	60·8	14·8	1·24	0·055	0·051	0·035
BLC:1(4)	25·5	70·9	9·42	2·11	0·050	0·048	0·048
BLB:1	0·9	9·03	4·98	3·80	0·053	0·040	0·118
BLH:3	15·6	31·2	3·93	1·65	0·154	0·126	0·179

[1] Thresholds are in quanta (507 nm) at the cornea and are calculated as described in BARLOW and LEVICK (1969a). Q_eSR is the average number of quanta per additional impulse. Dark lights are estimated from the maintained discharge and the slope of the responses to stimuli, using both the number of extra impulses per response period, and the variance of this number (see text). Multiplication factor is the ratio of variance to mean of the response. Estimates of overall quantum efficiency are explained in the text.

Other on-center units were as sensitive as this one, as shown in Table 1, but some had higher thresholds and quantum/spike ratios; however, in these cases there was usually a good reason for poorer performance, such as an ageing preparation, pulmonary infection, poor optics, or a much-prodded retina.

These results show that retinal ganglion cells are efficient and that averaging the responses of many neurons is not the only way that sensitivity and reliability can be achieved. Here, however, we are more interested in the retinal mechanisms subserving this great sensitivity. The quantum/spike ratios given in Table 1, column 4, are the ratios of the average

number of quanta delivered at the cornea to the average number of additional action potentials elicited (Q_cSR). We would like to know the fraction, F, of quanta that are absorbed effectively in retinal rods, which would tell us how many absorbed quanta are required per spike, Q_aSR. Previously we estimated that 25 per cent of quanta incident at the cornea were absorbed (BARLOW and LEVICK, 1969a); this was provisional and in the discussion we attempt a more accurate estimate, but if 2 quanta entering the cornea cause an average of one extra impulse, the possibility that each absorbed quantum causes more than one impulse must be seriously considered.

Statistical estimate of fraction effectively absorbed. HECHT, SHLAER and PIRENNE (1942) obtained important confirmation of their conclusion about the human threshold by means of the following statistical argument. If only 7 quanta are absorbed at threshold, then major fluctuations must occur in the actual numbers of quanta absorbed from a series of nominally identical flashes at a fixed, near-threshold, mean intensity. Thus, when flashes causing an average absorption of a quanta are given, a subject who sees the flash whenever 7 quanta or more are absorbed will see a proportion, P, given by the cumulative Poisson equation:

$$P = \sum_{c=7}^{\infty} \frac{e^{-a}a^c}{c!}.$$

The results of determinations of the proportions of flashes of various intensities that were seen fitted this equation, the best-fitting value of c varying from five to eight in different subjects. This indicated that at least 5–8 quanta played a part in causing the sensation, and it confirmed their estimate that 5–14 quanta (average) were absorbed from a threshold flash.

Since this small number of absorptions is spread over 300–500 rods, an individual rod must be capable of responding to a single absorption, and it is initially puzzling that one does not "see" when less than 5 quanta are absorbed. The results of HECHT *et al.* (1942) are consistent with the assumption of a weak "intrinsic retinal noise" that necessitates a multiple-coincidence criterion to avoid false positive responses in the absence of a stimulus (BARLOW, 1956). Obviously the existence of the maintained discharge supports this assumption and in the next section we use it to estimate the magnitude of the intrinsic noise or "dark light". However, the introduction of dark light introduces another parameter whose value, though determined from the experimental evidence, greatly influences the statistical estimate of the number of quanta absorbed, and it is this value that we are interested in at this stage. We have therefore used three methods of estimating the fraction effectively absorbed (quantum efficiency). In the first, corrections for the maintained discharge are applied in as straightforward a manner as possible; in the second, no correction is applied but conditions are selected where it makes little difference; in the third, a value for dark light is calculated from the results.

Variance of PND's. Consider the two pulse number distributions shown in Fig. 1. The left hand distribution has a mean $\bar{N}_o$ and variance $V(N_o)$; as a result of the absorption of Q_a quanta the mean became $\bar{N}_o + \Delta\bar{N}$, and the variance $V(N_o + \Delta N)$. These four values are readily determined experimentally. Figure 2 shows that ΔN is proportional to Q_a for weak flashes of near-threshold intensity; that is, increments of response are being added independently. Consequently, the variance of the impulse number distribution during the response period, $V(N_o + \Delta N)$, is the sum of $V(N_o)$ and $V(\Delta N)$, the variances of the maintained discharge and of the number of extra impulses added by Q_a. Thus, as well as obtaining $\Delta\bar{N}$ directly from the difference of the means, we can also estimate $V(\Delta N)$ from the difference

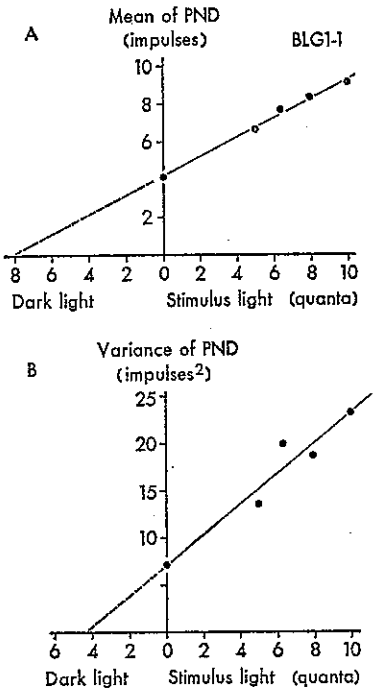

FIG. 2. (A) Relation between mean number of spikes per counting-period (200 msec) and mean stimulus level in units of quanta (507 nm) at cornea Q_c. Extrapolation of the linear relation (dashed line) yields level of "dark light", R_c, in terms of the equivalent number of quanta 507 nm) at the cornea. (B) relation between variance of number of spikes per counting-perod and mean stimulus level. Extrapolation to the left yields a second estimate of dark light.

of the variances of the two distributions. It is, however, essential to make sure that the relation between ΔN and Q is linear over and beyond the value of Q employed. As expected, the additional variance is reduced when saturating type nonlinearity develops, and this can cause very misleading underestimates of $V(\Delta N)$.

Suppose that an absorbed quantum causes, on average, m extra impulses; then $\Delta N = mQ_a$. Because quantal absorption follows Poisson statistics $V(Q_a) = Q_a$, and $V(\Delta N) = m^2 Q_a$ if transduction adds no more variance; but this possibility cannot be excluded, hence we can only say $V(\Delta N) \geqslant m^2 Q_a$, and $V(\Delta N)/\Delta N \geqslant m$. Eight estimates of $V(\Delta N)/\Delta N$ from pulse number distributions of the type shown in Fig. 1 yielded mean and S.E. values of 3·01 $\pm$ 0·33, as shown in column 5 of Table 1. Thus m has a value up to three: as many as three impulses may result from the absorption of a single quantum. If it is as great as 3, the fraction of quanta absorbed is $1/Q_c SR \times m = 0{\cdot}165$, as shown in column 6 of Table 1. Corresponding figures are shown for other units.

Probit analysis. In this method a probit regression line (FINNEY, 1947) is calculated for the probability of reaching or exceeding a criterion number of impulses as a function of the square root of stimulus strength. The quantum efficiency is one quarter of the square of the slope of the regression line (BARLOW, 1962), and estimates of sampling error, homogeneity, and confidence limits of the estimate can be derived. In this approach no allowance is made for dark light, and this leads to low estimates at low criterion numbers of impulses. Saturating nonlinearity of response causes underestimation at high values, and

the figures quoted in column 8, Table 1 are for intermediate values where the confidence limits were reasonable.

The third method of calculating quantum efficiency requires an estimate of the dark light.

Dark light. So far we have made use only of the mean and variance of the distributions (PND's) of the number of pulses in the maintained discharge and response. Clearly more information is available, but it is difficult to extract it without making more specific assumptions about the nature and magnitude of the dark light, which is here defined as the intrinsic source of retinal excitation that causes the maintained discharge of on-centre units in complete darkness. A simple assumption which fits the psychophysical evidence is that events occur in the receptors that are indistinguishable centrally from the absorption of a quantum of light (BARLOW, 1956, 1957). Thus the dark light can be expressed as the number R_a of random independent events that would be confused with the absorption of a quantum of light, or by the amount of light at the cornea R_c which would cause this average number of absorptions. An estimate of this figure can be obtained from Fig. 2 by extrapolating the line of added impulses backwards to zero impulses. This occurs at abscissa value −8·2, indicating that the excitation when the eye is in complete darkness is equivalent to 8·2 quanta at the cornea per analysis period (200 msec.) In a similar way, the variance can be accounted for by a light of 4·4 quanta at the cornea. These figures do not agree well with each other, and we shall return to this later when considering an anatomical explanation for our results (see p. 99 of this article); for present purposes an intermediate value of 6·5 will be adopted, and the multiple response hypothesis will be tested. Table 1, column 2, gives the average intercept for other units.

Cumulative Poisson method. First consider the specific hypothesis that when 1 quantum is absorbed three impulses result, for 2 quanta, six impulses, and so forth. Accordingly, if the probabilities of the ganglion cell response equalling or exceeding three impulses, six, nine

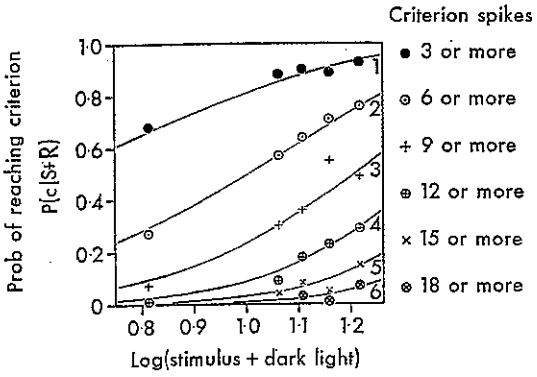

FIG. 3. Frequency-of-"seeing" curves for a retinal ganglion cell (BLG:1R1). For each set of data points, "seeing" is interpreted as the attainment of the corresponding criterion number of spikes indicated against the symbol on the right. To facilitate theoretical comparison, the data are plotted against log of stimulus strength (S) added to dark light (R_c) obtained from Fig. 2, and expressed as quanta at the cornea. The continuous curves are calculated for an ideal detector handicapped by the same amount of dark light (equivalent to 6·5 quanta at cornea per analysis period, 200 msec). Each curve corresponds to the attainment of the criterion number of quantal absorptions shown at the right-hand end. The curves have been fitted to the data points by a lateral shift which on the log scale corresponds to the fraction of corneal quanta effectively absorbed, in this case 16·6 per cent.

etc. are plotted as a function of log $(Q_e + 6{\cdot}5)$, these should be fitted by cumulative Poisson curves for criteria of 1, 2, 3 etc. quantal absorptions, plotted against the log of the average number of absorptions. The positioning of these curves relative to the scale of log $(Q_e + 6{\cdot}5)$ for the experimental points will depend upon the proportion of quanta at the cornea that are absorbed, and enable an estimate of quantum efficiency to be obtained.

Figure 3 shows results for 1 unit plotted in this way. Once the choice of three impulses per quantum has been made and the dark light has been determined, the only freedom in fitting the set of curves to the points is the lateral shift, and in this case the optimal position corresponded to 16·6 per cent absorption. This is in good agreement with the value of 16·5 per cent obtained by the other method. Corresponding figures obtained by applying the above procedure to other units are shown in column 7, Table 1. Generally, the fitting of cumulative Poisson curves to the experimental points after adjustments for dark light and multiplication factor gave good agreement, and isolated bad points or groups of bad points could often be explained by reference to the original protocol (e.g. unit lost and regained). For the last 4 rows, however, the fit was rather indifferent.

ROC curves. COHN (1969) has used signal detection theory to analyse the response of "off" units of the frog's tectum. A "receiver operating characteristic" (ROC curve) is obtained by plotting the probability of reaching a certain response criterion with one stimulus against the probability of reaching the same criterion with another stimulus (or a blank). For trains of impulses, the natural criteria are one or more impulses, two or more, three or more, and so on, and such ROC curves are plotted in Fig. 4, and also in Fig. 1. In addition points are plotted for an ideal detector with criteria of 1 or more quanta, 2 or more quanta, 3 or more quanta, etc., calculated on the assumption that 18 per cent of quanta are absorbed and there is a dark light of $R_e = 6{\cdot}5$. Clearly the points fit the curves well, and three, six, nine etc. impulse criteria lie close to the criteria for 1, 2, 3 etc. quanta. These points are also marked on the ROC curve of Fig. 1. In the present case the responses are linearly related to the stimulus, but, as Cohn points out, an important feature of this type of analysis is that

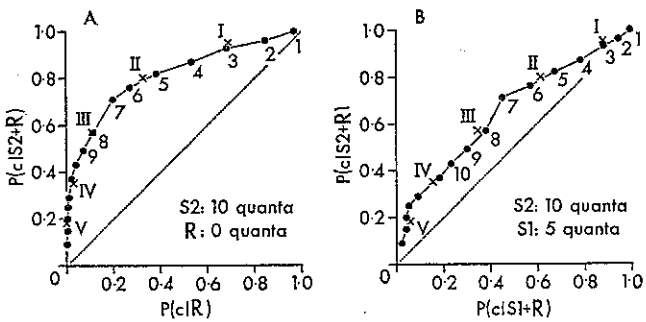

FIG. 4. ROC ("receiver operating characteristic") curves for experiment of Figs. 1–3. (A) Ordinate is probability that a criterion of at least c spikes will be attained in the presence of stimulus S2 (10 quanta at cornea) plus dark light (R_e); abscissa is the probability of attaining the same criterion in the presence of zero stimulus (dark light alone). Points corresponding to various criterion numbers of spikes ("operating points") are marked by Arabic numerals. The extent to which the curve deviates from the dotted "chance diagonal" measures the effectiveness with which the two stimulus conditions can be discriminated by observation of the ganglion cell output. Crosses and Roman numerals are points and corresponding criteria (quanta absorbed) for an ideal detector handicapped by a dark light equivalent to 6·5 quanta at the cornea, as well as a fraction effectively absorbed of 18 per cent (B) similar to (A), but for the discrimination of a stimulus of 10 quanta at cornea from one of 5.

it can equally well be performed when the responses are non-linearly related to stimulus intensity. In the present case the ROC curves of Figs. 1 and 4 provide a nice confirmation of conclusions reached by other means.

DISCUSSION

The results of Table 1 show that the absolute threshold of a single ganglion cell in the cat's retina is often as low as 10 or 20 quanta of optimal wavelength light (507 nm) at the cornea. Some of the easily overlooked conditions necessary for getting high sensitivities are: selection of ganglion cells near the area centralis; use of small, well-focused stimuli; avoidance of deep anesthesia or ones with longlasting after-effects; tip-top condition of optics, retina, circulation, and respiration. These factors can readily explain differences between the current results, our own earlier results, and previous reports. The behavioural threshold for the whole cat is likely to be somewhat higher than these figures for single ganglion cells might suggest because the large number of parallel pathways makes a higher criterion necessary; if the criterion is set to allow a 0·002 probability in each, there will be a high probability of a false positive response in at least one of them. For comparison, the human psychophysical threshold is about 6× higher than the cat ganglion cells, and the human is at a further disadvantage because his maximum pupil area is only one-third as large as the cat's.

These results establish without much question that single neurons can function with great sensitivity and reliability, and that single quantal absorptions are signalled reliably and efficiently by the retinal ganglion cells of the cat. There is another aspect of thresholds that the cat's retina tells us about; in the human, not only is there a requirement for 7 or more quanta, but also the requirement is least when the quantal absorptions occur in a small area and within a short time of each other. These limits to spatial and temporal summation have often been explained in terms of a coincidence mechanism, as suggested in different forms by HECHT, SHLAER and PIRENNE (1942), BOUMAN and VAN DER VELDEN (1947), BAUMGARDT (1953) and BRINDLEY (1963). Many have thought that this coincidence mechanism might be in the retina, but our results suggest that this is not the case. In the cat there is no sign of threshold-type non-linearity in the response of retinal ganglion cells at absolute threshold; any tendency to non-linearity has been of the saturating type. We are therefore led to believe that the discriminating mechanism that separates "seen" from "not-seen" operates more centrally.

To achieve high sensitivity to light one requires absorption of a large proportion of the available quanta, efficient generation of a neural signal, and a low noise level. These aspects will be discussed in turn.

Fraction of quanta absorbed. This can be estimated from what is known about receptors in other species as well as the cat, though it will become evident that there is great uncertainty. The factors that must be taken into account, together with our estimates, are as follows:

(1) Transmission of cornea and optic media. LUDVIGH and MCCARTHY (1938) found that only 51 per cent of light striking the cornea at 507 nm was transmitted to the retina in enucleated human eyes. Some more recent estimates have yielded higher figures, namely 92 per cent at 510 nm in rabbits (WEISINGER, SCHMIDT, WILLIAMS, TILLER, RUFFIN, GUERRY and HAM, 1956), and 80 per cent at 510 nm in bovine eyes (PITTS, 1959). However, these authors did not make clear how much of the forward-scattered light was included in the transmitted light they measured. BOETTNER and WOLTER (1962) found the indirect transmission, which includes the scattered light, to be almost double the direct transmission, and

their final figure for direct transmission is below 40 per cent at 507 nm in humans. On the other hand most of the scattering occurs in the cornea (DE MOTT and BOYNTON, 1958) and this reduced direct transmission to only 65 per cent in Boettner and Wolter's measurements. The cornea was probably in much better condition in our cats than in their enucleated eyes. Also transmission losses are higher in older animals, increase progressively after enucleation, and are likely to be higher in man than in a noctournal animal like the cat. We think the fraction striking the cornea that reaches the retina must lie in the range 50–85 per cent.

(2) Cross section of retinal rods. Some light reaching the retina will enter cones or the interspaces between receptors instead of rods. The figures of SCHULTZE (1866) suggest that cones do not occupy more than about 10 per cent of the space; allowing about another 10 per cent for inter-space, we estimate that between 70–90 per cent of the light reaching the retina enters the rods.

(3) The greatest uncertainty is in the absorption of light by the receptors. LIEBMAN and ENTINE (1968) say that the absorption per micron length at peak absorbance varies little from receptor to receptor, and MARKS (1965) and DOBELLE, MARKS and MACNICHOL (1969) agree with this. However, Liebman and Entine's figure for the optical density (0·016 per μ) gives an absorption almost double the figure (1·8 per cent per μ) derived from difference spectra by Dobelle, Marks, and MacNichol. There may be attenuation by factors other than the photosensitive pigment, so 1·8 per cent per μ is probably preferable for our purposes, but there is also great difficulty in specifying the length of the outer segments of cat rods, especially as they may be twice as long in the area centralis as in the periphery. SCHULTZE's (1866) figures indicate a value of 30–37 μ, but if the length varies from 25 to 50 μ, the absorption will vary between 36–60 per cent, and would be much higher if Liebman and Entine's figure was used.

(4) All our measurements were made on units overlying the tapetum, which has a reflectance of about 35 per cent at 507 nm (WEALE, 1953). If the absorption on single passage is 36 per cent, 23 per cent will be reflected and 8 per cent absorbed on the return passage leading to a total absorption of 44 per cent. If 60 per cent is absorbed on single passage the above reasoning gives a total absorption of nearly 69 per cent. We neglect the possibility that some light passing between the rods, or through the cones, may be absorbed in rods after reflection.

Taking all these factors into account our original estimate of 25 per cent for the fraction of quanta striking the cornea that are absorbed in retinal rods appears reasonable, but we can only feel confident that the true figure lies in the range 15–50 per cent. Applying this to the results of Fig. 1, it will be seen that the average number of quanta absorbed per flash was probably about one, but could have been as high as 2·5, equal to the average number of impulses evoked. One absorbed quantum probably causes several impulses, but we need support from statistical evidence.

Quantum efficiences and quantum/spike ratios. The different methods of calculating overall quantum efficiency agree reasonably well with each other, and show that different ganglion cells give results varying over the range 5–15 per cent, occasionally a little higher. This means that *at least* this percentage of quanta at the cornea effectively participates in generating the response. A higher percentage could be absorbed, but some might fail to bleach rhodopsin because the photochemical quantum efficiency of that step is less than unity, or any other step up to the generation of the impulses might degrade the efficiency from what might be expected from the estimated fraction absorbed. We cannot say what does this, but results such as those of Figs. 3 and 4 are consistent with a lower absorption

than we estimated, or a low quantum efficiency of bleaching, or of rod activation. Our results exclude any step or steps in the transduction that are less than 30 per cent efficient, for with 50 per cent absorption this would reduce the quantum efficiency below the measured 15 per cent. If only 15 per cent of quanta are absorbed, transduction must be 100 per cent efficient in some cases.

When these figures are considered in conjunction with quantum/spike ratios (corneal) it is hard to avoid the conclusion that more than one impulse usually results from a single effectively absorbed quantum. If the fraction absorbed was actually at the upper end of the estimated range, this would not be necessary, but the fact that the variance of the response is several times its mean would be unexplained.

It may be objected that if there is a multiplication factor that converts 1 quantal absorption into three impulses without adding any noise one would expect the resulting pulse number distribution to be multimodal, consisting solely of responses with zero, three, six, nine etc. impulses. We have not observed this, but do not feel it counts seriously against the multiplication hypotheses. First, the counting gate is not synchronized to the events causing the maintained discharge, and, as we shall point out later, the average multiplication factor for these events may not be the same as for the stimulus events when their spatial distributions are not the same. Second, if the multiplication was not noiseless but caused 3 ± 1, $6 \pm 1, 9 \pm 1$ impulses, then the gaps would be filled with relatively little increase in variance. Third, suppose the multiplication is very noisy, so that three, six, nine etc. are the average number of events produced by 1, 2, 3 etc. quanta, but the actual number occurring is a Poisson distribution with mean 3, 6, 9 etc., then it can be shown that the ratio of variance/mean of the resulting distributions is $m + 1$ instead of m, where m is the multiplication factor. A multiplication factor greater than 1 would still be needed in many cases.

From HECHT, SHLAER and PIRENNE (1942) we already knew that 1 quantum could excite a rod, but it seemed that several activated rods might be required to excite a ganglion cell. Now we know that the retinal mechanisms must meet more stringent demands, for a single activated rod must be capable of causing several extra impulses from a ganglion cell.

Retinal noise level. The second column of Table 1 shows the dark light, expressed as quanta at the cornea, required to explain the maintained discharge of on-center units in darkness. In the present experiments, it is quite clear that this, rather than a coincidence mechanism, is what limits retinal sensitivity, because it makes false responses inevitable if too small a number of impulses is accepted as signalling a real light. Considering the number of rhodopsin molecules poised to generate impulses if they are isomerized, these low figures for R_c clearly indicate that the rate at which they spuriously generate a response is exceedingly low, but figures for receptive field area are required to calculate an actual figure for the stability of the rhodopsin-rod excitation mechanism. Our results do, however, give interesting information about the distribution in time of the spurious excitations.

In our earlier work on the maintained discharge of the cat retina (BARLOW and LEVICK, 1969b), we were surprised to find that, in darkness, the variance of the PND often exceeded the mean by a factor of 2 or 3. Clearly the present finding that the variance of the response is often several times the mean is closely related; both facts can be explained by a single quantum causing several impulses, and by the "dark light" events behaving like quantal absorptions in this respect.

Again, in a scaling model to account for interval histograms (BARLOW and LEVICK, 1969b) we were puzzled that the scaling parameter derived from the maintained discharge in the dark (mean interval squared divided by variance) was less than 1. The idea of multiple

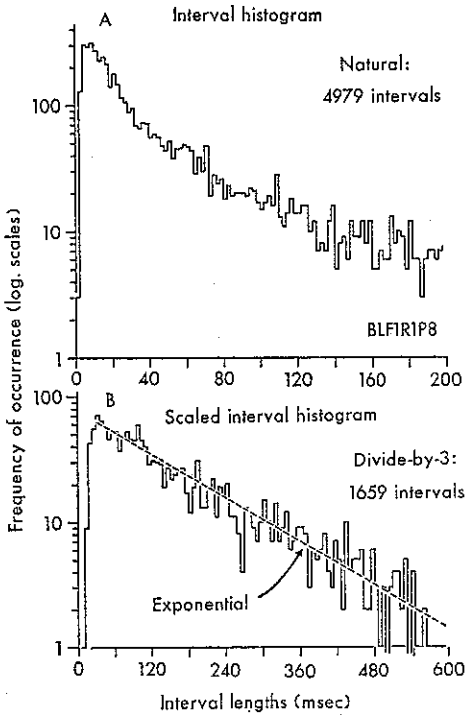

FIG. 5. Test of multiplication hypothesis. (A), standard interval histogram (2 msec bin width) of maintained discharge from on-center ganglion cell in darkness. (B), "scaled" interval histogram (6 msec bin width) resulting from ordered deletion of two out of every three impulses in the discharge, thus generating the distribution of sums of 3 adjacent intervals. The dashed line line is the best-fitting negative exponential in the range 48–588 msec obtained by minimizing the chi-squared statistic by iteration: final value = 61·47 with 60 deg of freedom, indicating a good fit.

spikes from single quantal events might explain this problem as well. A simple test of the hypothesis is illustrated in Fig. 5. At the top is shown the distribution of intervals between adjacent pulses in the dark discharge. There is a deficit of very short intervals near the vertical axis, possibly the result of relative refractoriness which will be ignored. With this log scale of ordinate, the concave upward shape is associated with a scaling parameter less than 1, and the test was to see how much pooling of adjacent intervals would be required to straighten the line and produce the negative exponential distribution characteristic of a Poisson process, the type of source presumed to represent the dark light. If each event of the Poisson process gave rise to two spikes within a short and variable interval, then one should be able to reconstruct the original Poisson process by combining the ganglion cell intervals in pairs before compiling the histogram, provided that one ignores the short-interval range where overlap of the multiplication process and refractoriness complicate the picture. In the example illustrated, it was necessary to combine adjacent intervals in sets of three before the distribution obeyed the negative exponential law (dashed line in Fig. 5, *B*). For the unit of Figs. 1–4, the combination of adjacent intervals in sets of 2 produced an equally good fit to that law. Thus the experimentally obtained histograms could, by this test, have been generated by a Poisson process causing three and two impulses respectively for each occurrence of a "quantum absorption-like event" in darkness.

To summarize, statistical analysis of the responses shows that in some cases at least 15 per cent of corneal quanta effectively participate in their generation, the maintained discharge looks as if it is generated by unitary events with the same statistics as quantal absorptions, and in many cases more than one impulse is generated by a quantal absorption or "unitary event". The following anatomical explanation for some of these facts led to further experimental tests.

An anatomical explanation. In searching for a reason why multiple responses might result from a single event in a receptor, one recalls a detail of the anatomy: a rod usually makes contact with several bipolar cells, and a ganglion cell usually receives input from several bipolars (MISSOTTEN, 1964; BOYCOTT and DOWLING, 1969). There is therefore a distinct possibility that several pathways to a ganglion cell could be activated by a single quantum

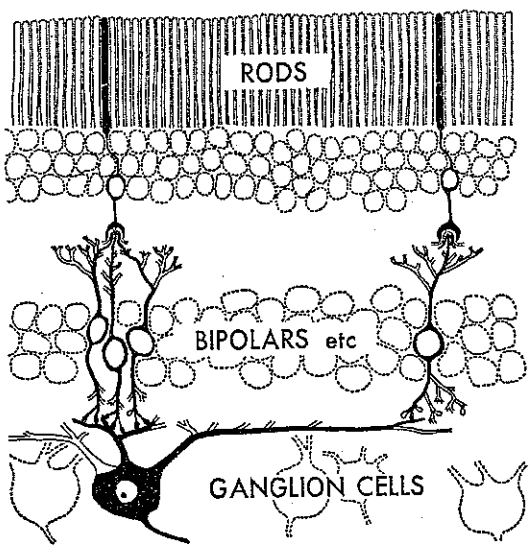

FIG. 6. Possible anatomical basis for multiplication hypothesis. A single receptor may communicate with a single ganglion cell by more than one independent bipolar pathway (shown in black) in the center of the latter's receptive field (left side of figure). Near the margin of the receptive field center (right), the multiplicity of pathways is likely to be reduced to one, and the bipolar signal may be attenuated in the ganglion cell dendrite.

catch, and if the ganglion cell gave an impulse for each activated bipolar the multiple responses could be explained. This is illustrated diagramatically in the left half of Fig. 6. Such multiple pathways are more likely in the centre of the receptive field; towards the edge of the central excitatory zone, a ganglion cell might be expected to pick up from no more than a single member of the bunch of bipolars activated by a single rod, and its influence would be attenuated by electrotonic decay along the dendrite as shown in the right half of Fig. 6. Thus multiple responses in the centre of the receptive field and the decline of sensitivity at the edge might both be explained anatomically. If this is correct, the ratio of variance to mean for a response elicited from the periphery of the receptive field should be reduced, and the ratio for a response from a uniform stimulus should also be less because of the contribution from the peripheral parts.

To test these predictions, the ratio of variance to mean was measured for the response to a small central spot, and to a displaced spot. The sensitivity in the periphery of the on-center zone was less, because it fell on a region of the receptive field less effectively connected to the ganglion cell, and as predicted the ratio of variance to mean of the response was greatly reduced. The ratio was also measured for a large spot covering the whole receptive field, and this was intermediate between the other two.

The anatomical explanation for multiple responses and a variable multiplication factor is obviously not the only one, but these results are compatible with it. They are not compatible with the variability of response being caused at a level later than that which causes variable sensitivity, e.g. the result would not have been obtained if variability was neural in origin, the receptive field was small with sharp borders, and the reduced sensitivity to a displaced spot resulted from light scattered to the centre.

The ratio for the uniform stimulus turned out to be nearly equal to the ratio for the maintained discharge. This is to be expected if the maintained discharge is caused by events indistinguishable from quantal absorptions occurring in receptors anywhere in the receptive field. Again, it is hard to reconcile the observations with an origin of the maintained discharge and variability of response at a more central point in the visual pathway.

SUMMARY AND CONCLUSIONS

(1) In many dark adapted on-center ganglion cells of the cat's retina, only 2 or 3 quanta (507 nm) at the cornea are enough to elicit an average of one extra impulse.

(2) From published results on ocular transmission, retinal histology and pigment absorption, we estimate that 15–50 per cent of quanta striking the cornea are absorbed by rods.

(3) The overall quantum efficiency of the cat's eye can be derived from the statistics of the response, and the results vary from about 5–17 per cent in different ganglion cells. For the sensitive ganglion cells, transduction in the retina is 30 per cent efficient if absorption is 50 per cent or 100 per cent efficient if absorption is 15 per cent.

(4) The variability of the response fits the hypothesis that absorption of a single quantum causes several extra impulses.

(5) Multiple impulses from single absorptions may result from multiple pathways from receptors to ganglion cells through bipolar cells. If so, receptors near the edge of the receptive field centre should not cause multiple responses; as predicted, the ratio of variance/mean for peripherally elicited responses was found to be lower than for centrally elicited responses.

(6) The maintained discharge in darkness has statistical properties similar to those of light-evoked responses; it behaves as though it results from random unitary events in the receptors each causing several impulses.

(7) At the absolute threshold single retinal ganglion cells transmit information on quantal absorptions in the receptors reliably, efficiently, and without threshold-type non-linearity. The mechanisms that set a discrimination level for separating what is seen from what is not seen must be situated more centrally in the visual system.

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Abstract—Under the best conditions, single ganglion cells of the cat's retina give one extra impulse (average) for less than 3 quanta (average) at the cornea. A stimulus containing about 15·50 quanta at the cornea is required to reliably modulate the maintained discharge.

Calculations suggest that when a single quantum is absorbed in the retina several extra impulses must be initiated. This is confirmed by the statistics of the response. For quanta absorbed in receptors in the periphery of the receptive field fewer impulses result, and this is confirmed by reduced variability of response.

Resumen—Bajo las mejores condiciones, cada célula ganglionar de la retina de gato genera un impulso extra (promedio) por algo menos de 3 cuantos (promedio) que llegan a la cornea. Un estímulo de aproximadamente 15,50 cuantos en la cornea es requerido para mantener una descarga modulada.

Los calculos sugieren que un cuanto absorbido en la retina podría iniciar varios impulses extras, lo que es confirmado por la estadística de la respuesta. Cada cuanto absorbido en receptores de la periferia genera menos impulsos, lo que es confirmado por la reducción en la variabilidad de la respuesta.