The absolute sensitivity of vision : can a frog become a perfect detector of light-induced and dark rod events?

Donner, K.

The Absolute Sensitivity of Vision: Can a Frog Become a Perfect Detector of Light-Induced and Dark Rod Events?

Kristian Donner

Department of Zoology, Division of Physiology, University of Helsinki, SF-00100 Helsinki, Finland

Abstract

Isolated retinal rods are known to spontaneously produce electrical "dark" events which appear identical to those associated with real photoisomerizations. Conceivably, this intrinsic noise at the very input to the visual system constitutes the "dark light", which sets an ultimate limit to the detection of weak lights in darkness. The performance of single neurons at different levels in the dark-adapted amphibian retina is shown to be consistent with this notion: (1) in the distal retina, horizontal cells display a dark noise of appropriate power in the relevant frequency band; (2) at the retinal output, the "best" ganglion cells perform as if their detection of dim flashes were limited by a dark light consistent with measured rates of dark rod events; (3) the proportion of such ganglion cells is sufficient to ensure that behavioural performance could in principle reach the same quasi-perfection.

Is the absolute threshold of vision essentially determined by a couple of inexorable limiting factors, impossible to improve on? Or is it set by a variety of limitations accumulated over the multiple stages of neural processing, so that no factor can be singled out as dominant — whereby the absolute threshold might even be subject to a significant degree of plasticity? Age-old belief would have it that sensitivity could be trained like muscular power far beyond the normal limit. Prisoners who were kept for years on end in pitch-dark dungeons (a type of psychophysical experiment less often done nowadays) were thought to learn to "see in the dark". In 1844, Dumas granted this advantage to the Count of Monte-Cristo, although Goethe already in 1810 [1] expressed himself with some caution: "Bey Gefangenen, welche lange im Finstern Gesessen, ist die Empfänglichkeit der Retina so groß, daß sie im Finstern (*wahrscheinlich in einem wenig erhellten Dunkel*) schon Gegenstände unterscheiden".

Today the detection of weak light is understood as a statistical discrimination of signal from noise. It is clear that there is an *ultimate* limit to any such performance. One undisputed limiting factor is the "extrinsic noise", the quantal fluctuations of the light itself [2, 3]. Undisputed in principle is also the fact that there has to be *some* "intrinsic" noise connected with the phototransduction and transmission machineries of the visual system — perfect detector perform-

ance is an ideal that may be approached, but not quite reached. But how close to that limit is the functioning of normal dark-adapted vision?

1. Rod noise and the absolute sensitivity of vison

In 1980, Baylor, Matthews and Yau [4] found that single rod photoreceptors from the toad retina spontaneously produce discrete electrical events indistinguishable from those connected with real photoisomerizations; somewhat later Baylor, Nunn and Schnapf [5] showed this to be true of monkey rods as well. Since then, the particular hypothesis that these spontaneous events constitute the main part of the intrinsic noise limiting detection at the absolute threshold has held great appeal. In accordance with ideas originally put forward by Barlow [6], this "photon-like" noise has been tentatively associated with spontaneous isomerizations of rhodopsin molecules, with which its thermal parameters are consistent. If so, it appears inexorable in two respects: (1) it is difficult to imagine a molecule (photopigment) that would have the property of being isomerized by visible light, yet would be thermally entirely stable; (2) if the spontaneous event is really identical to the elementary event in photoreception, there can be no physiological means (such as averaging, filtering or thresholding) of subsequently separating "real" from "false" photon signals.

There is considerable (bio-)logical beauty in the notion that such unavoidable noise at the input to phototransduction should be the decisive factor limiting the performance of the whole organism. Surely, evolutionary pressure would act to neutralize other sources of intrinsic noise down to that order of magnitude.

My presentation will therefore be framed in relation to the following particular hypothesis (H_0):

Under appropriate conditions the detection of a weak light signal in darkness is limited by spontaneous isomerization-like ("dark") rod events of the type and frequency described by Baylor et al. [4, 5].

It is worth noting that this is a strong hypothesis, since even the rods themselves produce other noise ("continuous" dark noise, Johnson noise: cf. Ref. [4]) which, in terms of sheer noise power in the relevant frequency band, is in the same order of magnitude as that due to the discrete dark events.

Unfortunately, the experimental evidence in support of this hypothesis does not live up to its *a priori* attractiveness. The case wholly rests on an approximate quantitative agreement between, on one hand, the measured rate of spontaneous rod events and, on the other hand, the detection-limiting noise at the output, the psychophysical "dark light"* of

---

* The "dark light" ("Augenschwarz" or "Eigengrau") as introduced by Fechner in 1860 is a concept originally extrapolated from measurements of the minimum intensity *difference* that can be detected, e.g., when presented as an intensity increment against a steady background of light. Such an "increment" threshold falls monotonically when background intensity is decreased, so it was natural to regard its final level, the absolute threshold, as set by an inner ("dark") background light which could not be turned off. Rose [7] and de Vries [8] in the early 1940's suggested that the detectability-reducing action of weak background lights is due to the quantal noise they introduce (see below). Following them, Barlow [6] suggested the idea that the dark light could be treated as an equivalent rate of photon-like noise events.

humans. However, the wide dispersion of dark light estimates makes it more correct to say only that the rod event rate is not inconsistent with these. Estimates obtained as limiting values from discrimination threshold experiments are listed by Barlow [9]. Expressed as equivalent numbers of 507 nm quanta (referred to the cornea) per second and square degree, the dark light estimates he considers most reliable, his own and those of Aguilar and Stiles [10], range from 630 to 3200 and 200 to 1300, respectively. Older experiments point to some 6000 [11], or 500 [12], or 2500–16 000 [13]. More recent frequency of seeing experiments (see below) suggest 400 [14] or 50–200 [15]. These are to be compared with the rod event rate measured in monkey by Baylor *et al.* [5], 0.0063 per rod and second, which by their own conversion factor would give about 670 q_{507} s^{-1} deg^{-2} incident at the cornea. At least it is evident that many subjects have been affected by limitations significantly more severe than that.

More disturbing is the conflicting evidence from retinal ganglion cells, the cells through which all information from the rods to the brain has to pass. As such, it is not surprising that ganglion cells in many situations may be affected by additional noise from sources more proximal than phototransduction (e.g., Ref. [16]). However, from the maintained discharge and spike/quantum ratio of cat ganglion cells, Barlow, Levick and Yoon [17] have estimated the underlying noise to be an order of magnitude *lower* than that expected from the measured rate of rod events in toad and monkey. Clearly, if the rod and ganglion cell values had both been obtained from one and the same species (and are assumed to hold for the intact organism), this would not be consistent with the notion that the spontaneous rod events are indistinguishable from real photoisomerizations. And, on the other hand, if human and cat ganglion cells are alike, it would mean that the psychophysical dark light is predominantly central in origin, as Barlow [18] has been led to suggest.

2. The hypothesis "detection limited by dark rod events" studied in frogs and toads

The experiments to be discussed aim at an evaluation of hypothesis H_0 in toads and frogs, whose visual system offers considerable advantages in this kind of study. Firstly, their absolute sensitivity to light is very high. Toads catching prey at night still snap at light levels so low that the human observer no longer sees them, only hears them [19]. Their sensitivity is now being quantitatively measured in behavioural experiments in Helsinki and Moscow (Aho, Donner, Hydén, Olesen Larsen, Orlov and Reuter, in preparation). Secondly, the spontaneous events in isolated rods have been most thoroughly studied in one toad species (*Bufo marinus*) [4]; by using this and closely related species, one avoids much uncertainty regarding species differences. Thirdly, the visual system of these animals is directly accessible to electrophysiological study at all levels.

Here, I shall be specifically concerned with the question whether signal transmission from rods and up to the *retinal output* fulfils a necessary condition for H_0 to be true:

At every level there must be at least some cells in which the discrete rod events constitute the main component of the detection-limiting noise.

To this end, I shall consider the performance of single neurons at two retinal levels; the horizontal cells in the distal
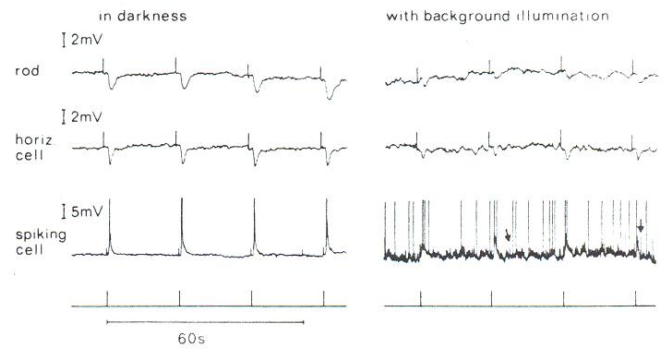
Fig. 1. Intracellular recordings from three levels in the toad retina: a rod, a horizontal cell and a spiking cell (probably a ganglion cell, but possibly an amacrine cell; spikes truncated to 40%). *Left*: in complete darkness, *right*: under steady full-field backgrounds (1.83 Rh* s^{-1} for the rod and spiking cell, 0.92 Rh* s^{-1} for the horizontal cell; Rh* denotes one photoisomerization per rod.) These backgrounds caused steady hyperpolarizations of -1.5 and -10 mV in the rod and the horizontal cell, respectively, and a 4 mV depolarization in the spiking cell. A 0.35 mm spot was flashed on the receptive field of each cell at 22 s intervals (as indicated under the recordings). In the rod recording, the flash intensity corresponded to an average of 2 Rh*, in the recordings from the horizontal cell and spiking cell to 0.3 Rh*. The upward "spikes" just preceding the responses in the recordings are pulses for amplitude calibration. From [21].

retina, in direct contact with the rods, and the ganglion cells, which represent the sole output of the retina. The results to be discussed are based on intracellular recording of membrane voltage in the toad *Bufo marinus* and extracellular recording of action potentials in the frog *Rana temporaria*, in both species from the eyecup preparation. They are mainly drawn from work done together with David Copenhagen (San Francisco) and Christel Hydén and Tom Reuter (Helsinki) [20–22]. Descriptions of techniques can be found in Refs. [21] and [22].

3. Noise in the distal retina: horizontal cells

All direct knowledge of dark rod events comes from a highly unphysiological preparation, single rods drawn into recording pipettes [4, 5]. Characterizing the noise of neurons in the distal retina therefore serves not only the evaluation of H_0. From another point of view, it helps to resolve two even more fundamental questions, namely (1) whether those events are a physiological reality in the intact retina, and (2) whether they are synaptically transmitted onwards. If so, it should be possible to find noise of the appropriate types and power in cells receiving their input directly from the rods, e.g., in horizontal cells.

Fig. 1 gives a general idea of how photoisomerization signals are transmitted and summated in the toad retina. It shows intracellular voltage records from three main levels: a rod (first order) cell, a horizontal (second order) cell, and a spiking (third or higher order) cell, in the dark (left) and under a weak steady background light (right). All records comprise flash photoresponses in the linear range (i.e., to weak stimuli, where response amplitude depends linearly on the number of photoisomerizations [25–27].

First consider the "dark" records. *Rods in situ* are extensively coupled electrically [26–28], therefore one should not expect to see any discrete isomerization-like events in one rod. An event arising in a single rod leaks out and becomes distributed as a low-amplitude signal in a great number of

rods. Still, the shape of a single event, unseen in the figure, is faithfully reproduced by the (much larger) flash responses as long as these stay in the linear range [29]. *Higher-order* neurons (the horizontal and spiking cells) at low intensities of stimulation behave approximately as devices linearly summing the signals from all rods within a certain area, the receptive field [22–24]. This means that a single rod isomerization event is reassembled as a discrete signal from the ensemble of rods on which it had been spread. But one should still not expect to discern single events in a horizontal cell record like that of Fig. 1: this cell, summing from some 340 rods at a rate of 0.028 events per rod and second (the expected rate of discrete rod events at 20°C, see Refs. [4, 21]), would "see" about 10 such events per second. Each event would have the same slow time course as the flash responses shown, but only about 1% of their amplitude, so the visible outcome would be no more than a noisy baseline.

This is precisely what the *background light* (right-hand) records illustrate: the effect of a steady low rate of isomerizations in the rods is to add a certain type of noise. Compared with the "dark" situation, the rod and the horizontal cell records clearly display a strengthened noise component in a frequency band roughly corresponding to that of the flash responses. It is subjectively quite evident that the superimposed flash responses become, as a consequence, much more difficult to detect. (In the spiking cell, the noise takes the form of increased maintained firing, which is usually transient in nature and won't be considered here.)

The impression that the frequency characteristics of the background-induced noise component in the horizontal cell are similar to those of the flash responses can be tested by comparing "dark" and "light" noise power spectra. In Fig. 2, a Fourier transform has been performed on stretches of noise (without flash responses) recorded in darkness and against a weak background. The resulting spectra are shown in Fig. 2(b). Subtraction of the "dark" spectrum from the "background" spectrum isolates the background-induced component in the form of a difference spectrum [Fig. 2(c), *squares*]. The *continuous curve* in Fig. 2(c) is a theoretical power spectrum generated from the waveform of small flash responses mathematically simulated by a Poisson model [25]. The good fit supports the idea that the background-induced noise is composed of events similar to dim-flash photoresponses.

The equivalent dark event rate of horizontal cells. Now, it is possible to proceed one step further. From Fig. 2(b) it can be seen that even the "dark" spectrum holds considerable extra noise power in the very same low-frequency band that was isolated by the difference spectrum. Assuming that his component, too, arises entirely out of isomerization-like rod events with the kinetics of dim flash responses, one can calculate the rate at which such assumed events must occur in order to yield the recorded spectral densities [4, 21]. In the cell of Fig. 2, such a calculation gives a "dark" event rate of 0.037 per rod and second. Since the calculation is based on *all* the noise in the relevant frequency band, it is in fact rather remarkable that the reported rate of discrete rod events (0.028 per rod and second at 20°C) would account for more than 75% of this calculated rate. The results obtained from two other horizontal cells analyzed in the same way were entirely similar.

The significance of this is twofold. Firstly, it suggests that the dark rod events are a physiological reality in the intact retina. Secondly, at the level of second-order neurons the record is still such that if it were appropriately processed (filtered, etc.), *signal detection* would be limited by that noise.

4. Detection-limiting "dark" noise in retinal ganglion cells

The retinal ganglion cell codes a graded voltage input into discharges of discrete spike potentials, which constitute the retinal output. Frog and toad ganglion cells usually operate at a very low level of maintained discharge (on the order of 1 spike/min or less), so the occurrence of one or more impulses within a couple of seconds after a stimulus is a significant response. These cells really perform a detection task, which can be profitably studied by procedures traditionally used in human psychophysics. The frog ganglion cell is a subject who answers "seen" by giving one or several spikes and "not seen" by remaining silent.

The purpose here is to estimate the noise interfering with the detection of a light stimulus. In the following I shall consider experiments applying two useful methods basically borrowed from psychophysics. One employs the threshold-raising effects of weak *background* lights, the other is to record *frequency of seeing* functions.

4.1. *Some concepts*

Summation area and summation time. In these experiments, the ganglion cell is modelled as a counter of isomerizations and isomerization-like events occuring within a certain summation area (the receptive field) and time. It is assumed that the cell sends off one more more impulses if and only if the count within one such summation time exceeds a fixed threshold criterion c. Since the dark events in rods, like full-field background lights, represent rates of events over the whole retina, the results will obviously depend on how accurately the summation parameters are determined. These problems are considered in detail in Refs. [22–24]. *Spatial summation* is here measured as a sharply delimited summation area A_s within which all events are summed with equal weight, while events falling outside that area have no effect on the cell. A_s is defined as the ratio of the *integrated* spatial sensitivity distribution of the cell to the *peak* of the distribution, and experimentally determined by dividing the threshold intensity of a large stimulus by that of a very small stimulus centered on the point of peak sensitivity of the receptive field. *Temporal summation* is similarly measured as a sharply delimited summation (or integration) time t_i, within which all events are summed with equal weight and are therefore irresolvable in time. t_i is experimentally obtained as the ratio of the threshold quantity of light (quanta) to the threshold flux of light (quanta/second) measured with spatially coextensive brief-flash and on-step stimuli, respectively. (This is equivalent to finding the ratio of the *integrated* dim-flash response to the *peak* amplitude of the response.)

The event *rates* due to background lights or dark rod events (events/mm² s) can now be translated into *numbers* of events determining the statistics at the ganglion cell. This is achieved simply by multiplication with $A_s t_i$. It is often convenient to express the event rates in units of Rh* s⁻¹, where Rh* denotes one isomerization (or isomerization-like) event *per rod*. Then the conversion into numbers at the ganglion cell requires that spatial summation can be expressed in terms of
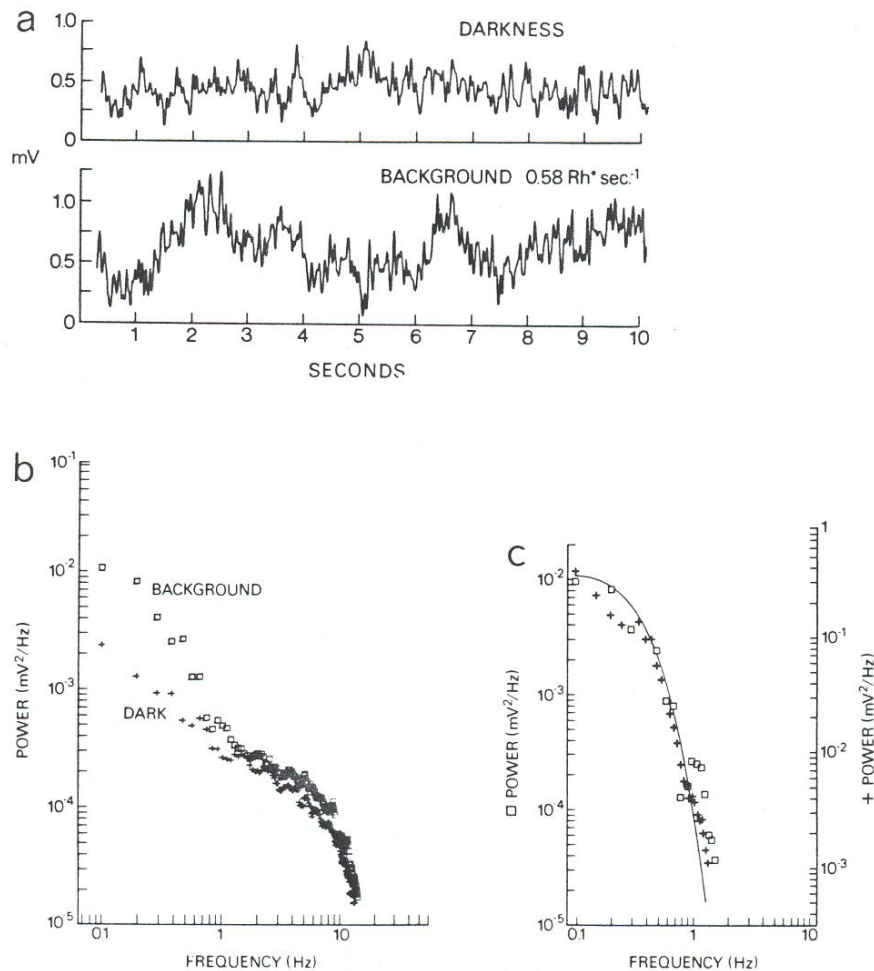
Fig. 2. Membrane voltage noise of a horizontal cell in the dark and under a steady background light ($0.58 \, \text{Rh}^* \, \text{s}^{-1}$). (a) Samples of intracellular recordings under the two conditions. (b) Power spectra of membrane noise in light (squares) and darkness (plusses). Spectral densities were calculated from twenty 10 s "dark" samples and seventeen 10 s "background" samples; average densities are shown (1024 pt FFT algorithm, five point smoothing above 1.2 Hz). (c) The *squares* show the difference spectrum obtained by subtracting the "dark" spectrum from the "background" spectrum of (b). The *plusses* show the power spectrum obtained from recordings containing responses to dim flashes (average of four 20 s samples low-pass filtered at 10 Hz, 24 db/octave). The left-hand ordinate refers to the noise difference spectrum, the right-hand ordinate to the flash response spectrum. The continuous line shows the power spectrum of theoretical flash responses, generated by fitting a "Poisson model" response [25] to the average flash response waveform during background. (The best fit was obtained with five stages of time constant 195 ms each.) From Ref. [21].

the number of rods within A_s, which is obtained through multiplication of A_s by the appropriate density of rods in the retina (*Rana temporaria*: mean $15\,700/\text{mm}^2$, [21]. *Bufo marinus*: mean $15\,000/\text{mm}^2$, [22]).

Expected rates of dark rod events. The results have to be judged in relation to the rate of discrete dark events in rods expected from the work of Baylor *et al.* [4] under the experimental conditions used here. The *Rana temporaria* experiments were conducted at the temperature 11.5°C, so the rate would be about $0.006 \, \text{Rh}^* \, \text{s}^{-1}$. This corresponds to about 94 isomerizations/$(\text{mm}^2 \, \text{s})$ or some 260 $\text{quanta}_{500\,\text{nm}}$ incident per second and mm^2 retina [21]. The corresponding figures for *Bufo marinus* at 20°C (as in the intracellular recordings considered above) are $0.028 \, \text{Rh}^* \, \text{s}^{-1}$, or 420 isomerizations/ $(\text{mm}^2 \, \text{s})$, or 950 $\text{quanta}_{500\,\text{nm}}$ incident per second and mm^2 retina [22].

The signal-to-noise ratio and noise-equivalent dark events in rods. If a light flash is calibrated to produce a mean number N of photoisomerizations within the receptive field of a cell, the actual number produced on each presentation will vary according to Poisson statistics [3, 6], whereby the standard deviation is equal to the square root of the mean. A signal-

to-noise ratio (*SNR*) can be defined as the mean flash signal divided by its standard deviation. The *SNR* of the flash stimulus (SNR_{in}) is then

$$SNR_{\text{in}} = N/\sqrt{N} = \sqrt{N}. \qquad (1)$$

This would also be the *SNR* of the flash *response* of a perfect noise-free detector. However, real phototransduction and transmission in the retina always entail some intrinsic noise, so the physiological *SNR* (SNR_{out}) of a cell's response cannot reach this upper limit; it must be somewhat lower. I shall turn this problem the other way round by assuming instead that we really have a perfect detector (with summation parameters A_s and t_i), and then ask: what would be the intensity I_x of a real background light that would degrade the *SNR* of the perfect detector's flash response to the value SNR_{out} actually observed in the physiological response of the cell? This amounts to referring all detection-limiting retinal noise to the *input*, expressing it as a *noise-equivalent* dark rate of isomerizations in rods (cf. Ref. [6]). The degree to which this rate agrees with that expected from the work of Baylor *et al.* [4] is a useful index of how well a cell or visual system conforms to hypothesis H_0.

If the mean number of events summed from the hypothetical noise-equivalent dark background within A_s and t_i is denoted X ($= I_x A_s t_i$), we obtain by definition

$$SNR_{out} = N/\sqrt{N + X} \qquad (2)$$

because the mean number of flash-induced isomerizations to be detected is, as before, N, while the standard deviation is now due to the Poisson variation around a total mean number $N + X$ events.

When a real *background light* is shining, the *SNRs* are affected also by isomerization events from that background. If the mean number summed within A_s and t_i is B, the *SNR* of the physical light stimulus (SNR_{inB}) becomes

$$SNR_{inB} = N/\sqrt{N + B} \qquad (3)$$

Expressing again the physiological output *SNR* (SNR_{outB}) using a noise-equivalent number of dark events X, we obtain for the background case

$$SNR_{outB} = N/\sqrt{N + B + X}. \qquad (4)$$

4.2. Desensitization by background lights

The estimation of dark event rate from measurements of how much dim backgrounds elevate the threshold intensity of a stimulus rests on two assumptions: (a) both in darkness and in light the detectability of the stimulus is limited by the random fluctuations in the total number of rod events, as expressed by eqs. (2) and (4); (b) the threshold response of the ganglion cell is of constant reliability, i.e., constant *SNR*. Further assume that the absolute threshold of the cell corresponds to a mean number N_t photoisomerizations produced by the stimulus, and that this threshold number is raised by a factor Z when a certain dim background is turned on. Let B_Z be the number of photoisomerizations that the cell sums from the background within its prevailing summation time and area. Then eqs. (2) and (4) give

$$N_t/\sqrt{N_t + X} = ZN_t/\sqrt{ZN_t + X + B_z} \qquad (5)$$

from which X can be solved.

In this way, equivalent event numbers X were estimated by eq. (5) in 17 frog cells. Rates per rod (Rh* s^{-1}) are obtained by dividing X for each cell by the number or rods in the receptive field and the summation time; the mean $\pm$ SE was 0.09 ± 0.04 Rh* s^{-1}. This, however, is not particularly illuminating, because cells differ by orders of magnitude. More interesting is the distribution of cells on different levels of dark event rates, plotted in Fig. 3 as a frequency histogram on logarithmic intervals. To facilitate comparison with hypothesis H_0, all values have first been normalized by the appropriate rate found in isolated rods, which means that they are expressed as multiples of 0.006 Rh* s^{-1}.

The leftmost class, with noise-equivalent dark event rates at the most two times higher than predicted by H_0, is the most interesting one. Containing 8 of 17 cells, it does suggest that at the absolute threshold a fair proportion of the cells really may be limited by noise the correct order of magnitude. But the histogram also shows that for many ganglion cells an account in terms of rod dark events is quite meaningless — they seem to be affected by much more powerful noise.

4.3. Frequency of response functions

The weakness of the above determination of X through eq. (5) is that the result is quite sensitive to inaccuracies in N_t
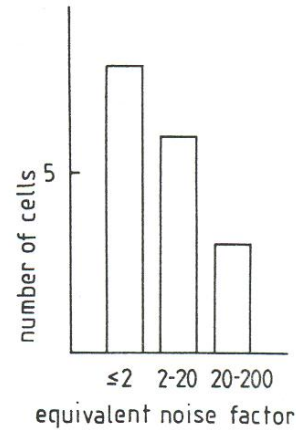

Fig. 3. Frequency histogram showing the distribution of frog ganglion cells ($n = 17$) on intervals of equivalent noise values, as determined from the threshold-raising effect of weak backgrounds. The equivalent rates of dark rod events thus found are here expressed as multiples of the rate 0.006 Rh* s^{-1} inferred from measurements in isolated toad rods [4].

and B_z. As a consequence, the dispersion is wide and as the mean is low, one even gets some negative estimates. More useful upper limit estimates are provided by another time-honoured psychophysical technique, the frequency of seeing (here, frequency of response or FOR) experiment. The protocol consists in presenting the subject with dim flashes of light at a few fixed mean intensities around the absolute threshold. The flashes are presented a great number of times in random order, and the proportion of presentations reported "seen" is recorded. A plot of these proportions against the mean number of photons (or photoisomerizations) delivered by the flash is a frequency of seeing curve [2, 6, 14, 15, 18]. In effect, this is a way of assessing the reliability of threshold responses.

When the ganglion cell is modelled as above as a counter of rod events, "seeing" (i.e., responding) if and only if the number exceeds a fixed criterion c, the Poisson statistics of these events requires that the FOR-curve be a cumulative Poisson curve. Assume that a cell gets a mean number N isomerizations from a flash and sums a mean number X dark events with these. On each particular presentation the total number actually counted will be $n + x$ varying around the mean $N + X$, and the probability (to be compared with the measured relative frequencies) that the number $n + x$ shall exceed the criterion c will be

$$P(n + x \geq c) = \sum_{k=c}^{\infty} [(N + X)^k/k!]\, e^{-(N+X)}. \qquad (6)$$

The shape of this function of N depends on its two parameters, c and X. The object here is to estimate X. That is possible, because the calibrations of quanta delivered and fraction leading to isomerizations are quite accurate in these experiments on opened amphibian eyecups; therefore $N_t = c - X$, which is the mean number of isomerizations from the flash producing responses on 50% of the trials, is always known with some confidence. This essentially leaves only one parameter, X, to be determined, which can be done by finding a maximum-likelihood value of X, i.e. that value for which the model expressed by eq. (6) gives maximum probability to the set of experimental data [21, 22].

Ganglion cells do not behave as noise-free detectors of photoisomerizations. In Fig. 4, the *continuous* lines show
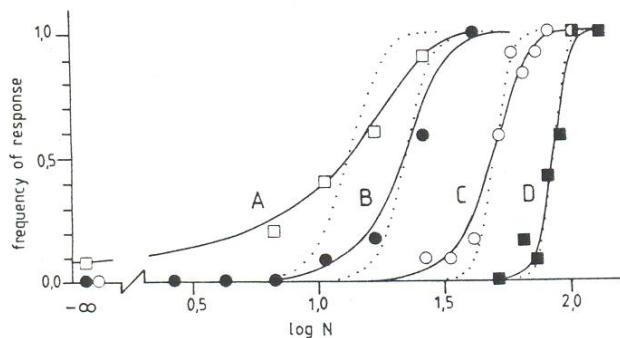
Fig. 4. Frequency of response functions recorded from four dark-adapted ganglion cells. Abscissa: flash "intensity", i.e., log mean number of photoisomerizations produced by the flash within the receptive field of the cell. Ordinate: the proportions of flash presentations that were followed by one or several spikes within a response window from 0.5 to 2.5 s after the flash. 12 flash presentations at each mean intensity. The 50% threshold for the four cells fall at flash intensities producing average numbers of photoisomerizations $N_t = 13, 22, 50$ and 85 respectively. The curves are cumulative Poisson probabilities according to eq. (5). The continuous curves are optimized fits, with equivalent noise values (left to right) $X = 90, 42, 150$ and 105 events summed within A_s and t_i, or expressed as event rates per rod and second 0.10, 0.009, 0.17 and 0.09 Rh*s^{-1}, respectively. The steepness of a curve is a good visual index of the reliability (the *SNR*) of a threshold response. The dotted curves are "zero-noise" models (with $X = 0$), illustrating the situation that would obtain if detection were limited only by fluctuations in the numbers of quanta absorbed (cf. [2] and eq. (1)). The stimulus was a 512 nm spot of 0.11 mm diameter and duration 1/15 s centered on the receptive field and always well contained within the summation area and time.

optimal (i.e., maximum likelihood) fits to four FOR experiments from frog ganglion cells. The *dotted* lines show for comparison the curves obtained with $X = 0$ in eq. (6), corresponding to purely "photon-limited" detection with no intrinsic noise. The four examples serve to illustrate several of the general conclusions that can be drawn from this type of experiments.

Firstly, the "zero-noise" curves are steeper than the optimal ones, illustrating the well-known fact that noise makes the curves shallower [6]. This is the basic reason why FOR-experiments essentially give upper limits to the noise: any extra variation (e.g., sensitivity drifts) flattens the curves further, leading to overestimated rather than underestimated equivalent noise.

Secondly, it really does make a highly significant difference what X-values are put into eq. (6). In three of the cases shown (A–C), the probability of the data is higher by a factor $> 10^4$ under the optimized fit than under the zero-noise model; in the fourth case (D) the probability factor is 17. This excludes the possibility that these ganglion cells perform as perfect (noise free) detectors of photoisomerizations.

Thirdly, in most cases the noise estimates from the optimized fits agree poorly with the values expected from the rate of dark events in isolated rods. In Fig. 4, the estimated noise-equivalent event rates are, respectively, 0.10 (A), 0.009 (B), 0.17 (C) and 0.09 (D) Rh*s^{-1}, i.e., only cell (B) is in approximate agreement with the rate expected (0.006 Rh*s^{-1}). The same thing can be expressed as follows: the probability of obtaining the experimental data is higher by factors 10^7 (A), 1.2 (B), 10^5 (C) and 9 (D) under the optimized fit than if the X value put into eq. (6) is based on the rate 0.006 Rh*s^{-1}.

Some ganglion cells do behave as required by H_0. The most important result of the FOR-experiments, however, is the
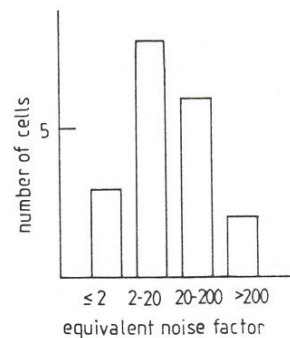
Fig. 5. Distribution of 19 frog ganglion cells on intervals of equivalent noise values, as determined by frequency of response recordings. Conventions as in Fig. 3.

positive conclusion that there really *are* a few ganglion cells such as cell (B) in Fig. 4, where performance is limited by a noise which can mainly be accounted for by the isomerization-like noise in isolated rods. Figure 5 summarizes dark noise estimates from FOR-experiments on 19 frog cells, displayed as in Fig. 3. As expected, the distribution is somewhat biased towards higher noise values compared with Fig. 3. Still, there are three cells (16%) in the class with equivalent dark event rates no more than twice that expected.

5. The absolute threshold of ganglion cells

Detection of very weak lights requires both reliability (sufficient *SNR*) and sensitivity. Obviously, a ganglion cell affected by powerful noise shouldn't put down its spiking threshold so much that it becomes congested by noise. On the other hand, a cell with a threshold far above available light levels is just as useless, however reliable. Unless the low-noise cells described above are also the most sensitive cells, the results cannot necessarily be taken to support H_0. Moreover, there has to be a sufficient proportion of such sensitive low-noise cells to ensure corresponding behavioural performance.

5.1. *Noise, sensitivity and reliability*

The threshold numbers of photoisomerizations N_t of the three cells affected by least noise in Fig. 5 were 7, 12 and 22. These represent reasonably, but not extremely, high sensitivities. In Fig. 6 are given the threshold numbers of photosomerizations N_t of 105 ganglion cells sampled by the microelectrode, plotted against their summation areas (on logarithmic axes; also note that threshold decreases, i.e., sensitivity increases, upwards in the Figure). In this sample, there are as many as 8 cells with $N_t \leqslant 7$, 19 cells with $N_t \leqslant 12$ and 33 cells with $N_t \leqslant 22$.

Indeed, the simplistic idea that ganglion cell sensitivities in general are strictly noise-determined, so that the dark-adapted threshold would always be set at the lowest level compatible with "sufficient" response reliability, is clearly untenable. In fact, threshold responses of insensitive cells are, on an average, much more reliable than those of sensitive cells: there is a strong positive correlation between log threshold (log N_t) and the *SNR* of threshold responses ($r = 0.707***$, $n = 19$). For example, referring back to Fig. 4, the threshold *SNRs* (eq. (2)) of the four cells were 1.3 for cell (A), 2.8 for cell (B), 3.5 for cell (C) and 6.2 for cell (D). In view of the fact that cells (A) and (D) had approximately the same estimated noise-equivalent event number (see legend
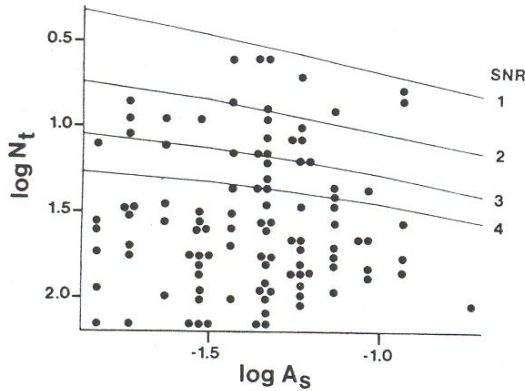
Fig. 6. Log threshold number of photoisomerizations (log N_t) as a function of log summation area (log A_s) for 105 dark-adapted ganglion cells. Note that N_t decreases (sensitivity increases) upwards in the figure. The family of full-drawn lines mark the dependence of log N_t on log A_s predicted if threshold would imply a constant-reliability detection of the flash against a background of dark rod events occurring at the rate 0.006 Rh* s^{-1}. Each of the four lines corresponds to one integer value (1, 2, 3 and 4) for that constant signal-to-noise ratio. (The figure includes only cells with thresholds below $N_t = 150$ photoisomerizations. With less sensitive cells, it is difficult to know, when insensitivity reflects "natural" properties rather than bad preparations. A subjective impression is that at the most 20% of dark-adapted cells may have thresholds higher than 150 isomerizations without pathological reasons or incomplete adaptation.)

to Fig. 5), it would appear that cell (D) could have "afforded" higher sensitivity.

Thus, many cells seem to be insensitive not because they are forced to by strong intrinsic noise, but for some other reason — maybe detecting dim flashes is none of their business. Interestingly, in Fig. 6 there is a suggestion that it is cells with somewhat larger receptive fields (i.e., cells which sum relatively much noise) that have the lowest absolute thresholds even in the strictest terms of *numbers* of photoisomerizations needed to reach threshold: 1°, of the 10 most sensitive cells ($N_t \leqslant 8$), 9 have a summation area $\geqslant 0.04$ mm^2 and only one cell is smaller (while the smaller cells totally make up one third of the cells); 2°, there seems to be a greater proportion of comparatively insensitive cells among those with smaller receptive fields (the lower envelope of log N_t in the Figure rises at higher log A_s values).

For most of the cells in Fig. 6 no proper estimate of the noise-equivalent dark event rate (e.g., from FOR-experiments) is available. By the following argument, however, it can be concluded that the "expected" rate of dark rod events (see Section 4.1) would alone be sufficient to importantly degrade the threshold response reliability of the most sensitive cells. Take, for example, the most sensitive 30% of the cells in Fig. 6 (33 cells with N_t ranging from 4 to 22). If there were *no* intrinsic noise, so that their *SNRs* would be given by eq. (1), these cells would show the following distribution of signal-to-noise ratios at threshold: no cells below *SNR* = 2, 10 cells between 2 and 3, 14 cells between 3 and 4, and 9 cells above 4. In contrast, under the hypothesis that the cells are limited by no *other* intrinsic noise than dark rod events occurring at the rate 0.006 Rh* s^{-1}, the distribution of threshold response *SNRs* (eq. (2)) would be as follows: 9 cells between 1 and 2, 11 cells between 2 and 3, 11 cells between 3 and 4, and only 2 cells above 4. (Into Fig. 6 have been drawn lines marking the integer *SNR* levels under this "minimum" noise hypothesis.) It is evident that the expected rate of discrete rod

events alone would constitute a major limitation to the reliable signalling of dim lights by this ensemble of sensitive cells. (At temperatures higher than the 11.5°C used here, this limitation would become much more severe, because the rate of dark rod events rises steeply with temperature [4].) This conclusion gains even more weight when it is appreciated that "natural" dark-adapted vision is concerned not with the detection of small, brief flashes, but of extended objects with relative permanence in time. Hence, under normal conditions detection at the absolute threshold is probably managed primarily by cells with wide spatio-temporal summation, which will necessarily also sum comparatively large numbers of dark rod events.

The general picture that emerges is that only some of the ganglion cells are tuned to high sensitivity, but in this subpopulation of cells the *reliability* of threshold responses could indeed be limited by the dark events known from single rods.

5.2. *Coverage factor of sensitive cells*

The proportion of such cells among those here sampled by the microelectrode is on the order of 20% (Figs. 5 and 6). Histological studies show, however, that cells with dendritic trees as large as any of the summation areas shown in Fig. 6 ($A_s > 0.01$ mm^2) make up less than 5% of the total number of ganglion cells (some 450 000 in the frog retina, [31] and Kock, Mecke, Orlov, Reuter, Väisänen and Wallgren, in preparation). It is by no means certain that the summation area of a ganglion cell could not exceed the size of its dendritic tree. Still, calculating a lower estimate, we have to accept the possibility that the very sensitive cells constitute less than 1% of all, i.e., some 4000 altogether. In Fig. 6, most of the sensitive cells were seen to have log $A_s \geqslant -1.4$, i.e., $A_s \geqslant 0.04$ mm^2. This would indicate coverage of a total area of roughly 4000 × 0.04 mm^2 = 160 mm^2. The mean diameter of the frog eyes used for the experiments reported here was 6.6 mm, giving the mean retinal area 68 mm^2. Accordingly, the very sensitive cells would have a coverage factor of 2.4, so every point in the visual field would be sampled by, on an average, at least two such cells. This semi-quantitative estimation is not taken as a claim that the sensitive cells form a distinct class. My purpose is only to show that the proportion of such cells encountered here is sufficient to support hypothesis H_0. Still, it is interesting that the existence of a distinct small subpopulation (2–5%) of large ganglion cells appears to be a common feature of vertebrate retinas (cat [32], dogfish [33, 34], carp [35]).

Between rods and ganglion cells lies an intricate processing network of interneurons determining the response properties of the ganglion cells (a versatile lot in frogs and toads). In laying down synaptic connections, stable or plastic on various time scales, the optimization for one task must often be detrimental for performance in another task. For instance, low-noise, ultra-sensitive detection of light under natural conditions entails a linear summation of excitatory signals from a large homogeneous receptive field. It seems reasonable to assume that this requires a certain degree of simplicity in the intervening network, making the cells less suitable for more specific feature detection. As it would probably be of pretty little advantage to have 50 or 60 cells (the total overlap factor in the frog) do the same simple light-detection at the same point in the visual field, it is a natural thought that the

majority of cells might not at all be concerned with that task. Instead, many cells may be subserved by a more complicated (partly inhibitory) neural network, imposing the penalty of lower sensitivity and higher equivalent noise in the simple task studied here.

6. Conclusion

If it is accepted that the animal's behavioural performance is determined by the ganglion cells best suited for each particular task, the results are consistent with hypothesis H_0. The detection of light in darkness by the most sensitive ganglion cells is really limited by an intrinsic noise which could largely be composed of the spontaneous isomerization-like rod events reported by Baylor *et al.* [4]. Thus, it is possible that the frog's performance in the simplest forms of behaviour such as phototaxis is actually close to an ultimate limit. One should realize that the conclusion can apply only to tasks analogous to those the ganglion cells here have been presented with. Other basic tasks already require much more information processing. For example, accurate prey-catching already asks for some stereopsis and judgment of motion. Little is known of how contradictory requirements of sensitivity vs. accuracy are balanced in such more demanding processing and whether there can be longer-term shifts of balance, e.g., to favour sensitivity during protracted residence in complete darkness. Longer-term rearrangements of synaptic connectivities are known to occur in the amphibian visual system at least in the form of seasonal changes [36, 37]. So it is not impossible that even the Count of Monte-Cristo could have had some aspects of his dark vision improved during his stay at the Château d'If.

References

1. Goethe, J. W., Zur Farbenlehre, p. 4, J. G. Cotta, Tübingen (1810).
2. Hecht, S., Shlaer, S. and Pirenne, M. H., J. Gen. Physiol. **25**, 819 (1942).
3. Baumgardt, E., Handbook of Sensory Physiology, Vol. VII/4 (Edited by D. Jameson and L. M. Hurvich) Ch. 2, p. 29, Springer-Verlag, Berlin (1972).
4. Baylor, D. A., Matthews, G. and Yau. K. -W., J. Physiol. (Lond.) **309**, 591 (1980).
5. Baylor, D. A., Nunn, B. J. and Schnapf, J. L., J. Physiol. (Lond.) **357**, 575 (1984).
6. Barlow, H. B., J. Opt. Soc. Amer. **46**, 634 (1956).
7. Rose, A., Proc. Inst. Radio. Engrs. **30**, 293 (1942).
8. de Vries, H., Physica **10**, 553 (1943).
9. Barlow, H. B., J. Physiol. (Lond.) **136**, 469 (1957).
10. Aguilar, M. and Stiles, W. S., Optica Acta **1**, 59 (1954).
11. Blackwell, H. R., J. Opt. Soc. Amer. **36**, 624 (1946).
12. Blanchard, J., Phys. Rev. **11**, 81 (1918).
13. König, A. and Brodhun, E., Gesammelte Abhandlungen zur Physiologischen Optik, p. 116. J. A. Barth, Leipzig (1903).
14. Hallett, P. E., J. Physiol. (Lond.) **202**, 421 (1969).
15. Sakitt, B., J. Physiol. (Lond.) **223**, 131 (1972).
16. Frishman, L. J. and Levine, M. W., J. Physiol (Lond.) **339**, 475 (1983).
17. Barlow, H. B., Levick, W. R. and Yoon, M., Vision Res. **11**, Suppl. 3, 87 (1971).
18. Barlow, H. B., Vertebrate Photoreception (Edited by H. B. Barlow and P. Fatt) Ch. 19, p. 335, Academic Press, London (1977).
19. Olesen Larsen, L. and Pedersen, J. N., Amphibia-Reptilia **2**, 321 (1982).
20. Donner, K., Hydén, C. and Reuter, T., Acta Univ. Oul. **A179**, 35 (1986).
21. Reuter, T., Donner, K. and Copenhagen, D. R., Neurosci. Res. Suppl. **4**, S163 (1986).
22. Copenhagen, D. R., Donner, K. and Reuter, T., J. Physiol. (Lond.) **393**, 667 (1987).
23. Donner, K., Acta Physiol. Scand. **131**, 479 (1987).
24. Copenhagen, D. R., Hemilä, S. and Reuter, T., Manuscript.
25. Baylor, D. A., Hodgkin, A. L. and Lamb, T. D., J. Physiol. (Lond.) **242**, 685 (1974).
26. Fain, G. L., Science (NY) **187**, 838 (1975).
27. Copenhagen, D. R. and Owen, W. G., J. Physiol. (Lond.) **259**, 251 (1976).
28. Schwartz, E. A., J. Physiol. (Lond.) **246**, 617 (1975).
29. Baylor, D. A., Lamb, T. D. and Yau, K.-W., J. Physiol. (Lond.) **288**, 613 (1979).
30. Barlow, H. B., J. Physiol. (Lond.) **141**, 337 (1958).
31. Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. and Pitts, W. H., J. Gen. Physiol. **43**, 129 (1960).
32. Wässle, H., Levick, W. R. and Cleland, B. G., J. Comp. Neur. **159**, 419 (1975).
33. Shibkova, S. A., Arkh. Anat. Gistol. Embriol. **60**(3), 21 (1971).
34. Stell, W. K. and Witkowsky, P., J. Comp. Neurol. **148**, 1 (1973).
35. Kock, J. H. and Reuter, T., J. Comp. Neurol. **179**, 535 (1978).
36. Ewert, J. -P. and Siefert, G., J. Comp. Physiol. **94**, 177 (1974).
37. Ewert, J. -P. and Siefert, G., Vision Res. **14**, 431 (1974).