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Old and New Results About Single-Photon Sensitivity in Human Vision

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Old and new results about single-photon sensitivity in human vision

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Abstract

It is sometimes said that 'our eyes can see single photons'. This article begins by finding a more precise version of that claim and reviewing evidence gathered for it up to around 1985 in two distinct realms, those of human psychophysics and single-cell physiology. Finding a single framework that accommodates both kinds of result is then a nontrivial challenge, and one that sets severe quantitative constraints on any model of dim-light visual processing. This article presents one such model and compares it to a recent experiment.

1. Prehistory

Soon after Einstein's light-hypothesis article, Lorentz realized in 1920 that, if light were indeed particulate, this would impose an ultimate limit for human visual sensitivity [1][1]. Physiologists had already undertaken rough estimates of the faintest flash that could be reliably seen; Lorentz converted their units into energy per flash, then divided by Einstein's result for the energy of one photon, and came up with the result that roughly 25–150 photons entering the human eye was enough to perceive a flash.

It is not surprising that evolution has made our eyes very sensitive. There is a tremendous advantage for a prey species to be able to forage in the nearly complete darkness of a moonless night, and a corresponding advantage for its predators. But the energy deposited by one hundred visible photons is many orders of magnitude smaller than the energy threshold for, say, touch receptors. Several questions then became urgent, for example (a) What is the best value we can measure for this limit; (b) Why has not evolution made us even better (why is not the minimal stimulus *one* photon); and (c) What do these statements even mean in the quantum world of light?

Concerning that last question, around the early 1930s Kubetsky invented the photomultiplier tube, a non-living instrument capable of detecting single photons. Figure 1 shows some data from a modern descendent of the PMT, and underscores the problem: even steady illumination turns out to consist of discrete blips, as Einstein had proposed, but the arrivals of those blips form a *random process* (usually a Poisson process [4]). When we attempt to assess the eye's sensitivity by presenting it with faint flashes of light, we therefore have no control over how many photons are delivered in any given trial. All we can measure is the *mean* rate of photon arrivals. Thus, even if we open a shutter for a known length of time, all we can state about the resulting flash is the *average* number of photons per flash, averaged over many trials (at least if we use common light sources like an incandescent light bulb, the Sun, or a laser). Brumberg and Vavilov appreciated this feature and attempted to measure statistical effects in threshold vision as early as 1933 [5].

[1] For further background, see [2, 3].

2. Hecht, Shlaer, Pirenne, van der Velden

Hecht and coauthors sidestepped the photon-uncertainty problem by reframing the question in probabilistic language [6]. They asked, 'for faint flashes that are as reproducible as possible, what is the relation between the *mean* number of photons delivered per flash and the *probability* $\mathcal{P}_{\text{see}}$ that a subject will report having seen it?' Figure 2 shows one dataset for one of their subjects. It is tempting at this point to find a mathematical function that passes through the data points, but that would be premature. Not only do the results depend on the age and other characteristics of the experimental subject; they also depend on the subject's *training*. Hecht and coauthors required their subjects to be quite certain before reporting a flash,

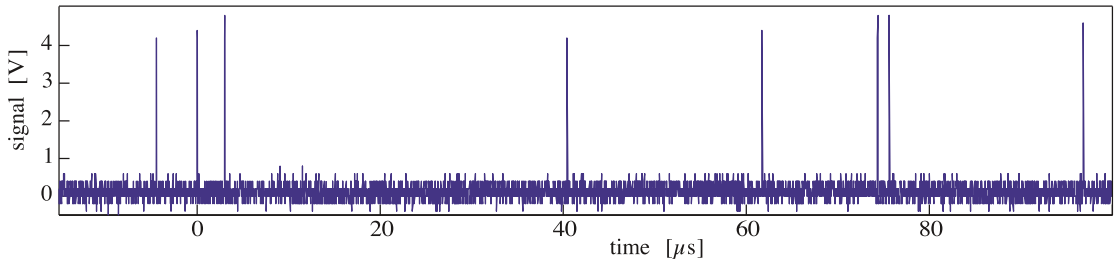

Figure 1. Time course of the signal from an avalanche photodiode detector exposed to dim light. The signal consists of similar blips, at random times. At a given wavelength, the intensity of the light corresponds to the mean rate of blip arrivals, not any attribute of the individual blips themselves. Data courtesy John Beausang.

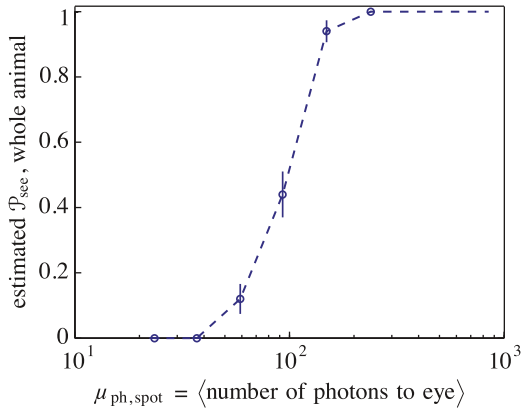

Figure 2. 'Probability of seeing'. Semilog plot of the fraction of 50 trials in which a human subject reported seeing a faint flash of light, versus the expectation of the number of photons delivered to the subject's eye in each flash. The error bars represent one standard deviation, based on the variance of a Bernoulli trial repeated 50 times. The dashed line is not a fit; it just joins the points. Data from [6].

that is, to 'never' give a false positive result, but taken literally, that instruction would require setting an infinitely high threshold. What the subjects actually did was therefore not very well defined. (Similar, independent measurements by van der Velden seemed to give greater sensitivity [7], probably because the injunction against false positives was not as strict [8].)

Despite that experimental weakness, Hecht and coauthors did obtain an extraordinary conclusion. They noted that that their subjects could reliably report seeing flashes with mean photon count as low as 100–200, even though each flash of light was focused to a spot on the retina containing several hundred rod cells. Assuming that each rod cell responds independently of the others, then the probability that any one of them caught two or more photons during the flash was very small. Hecht and coauthors concluded correctly that therefore, an individual rod cell must be able to generate a behaviorally relevant signal upon the productive absorption of just *one* photon.

This conclusion does *not* say 'our eyes respond each time they are presented with one photon'. Many photons are lost before they even arrive at the retina; others are absorbed there by something other than the signaling molecule. Even those that are absorbed by the right molecular species may not trigger any response (they can be 'nonproductively' absorbed). These reasons partly account for why subjects needed 100–200 photons in a flash in order to respond reliably. But additional experiments were required to get a fuller picture.

3. Barlow and Sakitt

Other lines of evidence suggested that a signaling molecule in rod cells, now called rhodopsin, was responding to light by photoisomerizing. H. Barlow reasoned that if that were the case, then there would also be events in which exactly the same isomerization occurred spontaneously due to thermal motion, *without* any photon. Rod cells would be unable to distinguish such events from genuine photoisomerizations, leading to a trickle of false-positive reports from each rod cell. Moreover, that trickle could potentially become a flood in low-light situations, where Hecht and predecessors had already shown that the outputs of many rod cells were pooled. Barlow made the simplest assumption, that this pooling just amounted to counting the single-photon signals generated by all rod cells in a summation region of the retina.

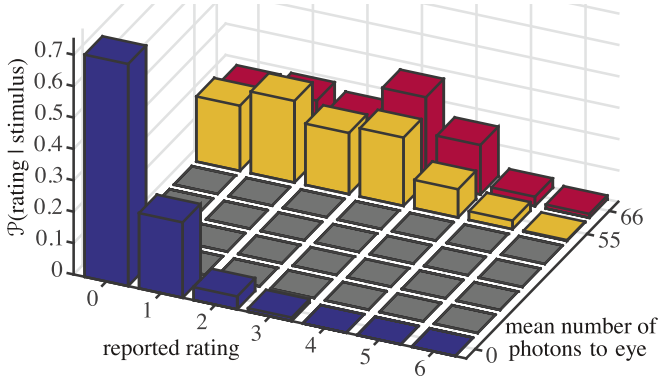

Figure 3. Sakitt's experimental data for one subject. Flashes of three different strengths $\mu_{\text{ph,spot}} = 0, 55$, or 66 photons were presented to the eye in random sequence, and the subject was asked to report their brightness as a 'rating' $r = 0, \ldots, 6$. For each flash strength $\mu_{\text{ph,spot}}$, the probabilities of each rating r were were estimated from the relative frequencies with which the subject gave each rating at each strength. This plot emphasizes that the experiment measured a function of two variables: $\mathcal{P}$(rating | stimulus). Data from [9].

Barlow proposed that some later stage of neural processing dealt with the false-positive problem by imposing a 'quorum' requirement[2]: some minimum number of photon-like rod cell signals must be received in a visual region, close enough in time, before the conscious mind would be alerted [8]. Barlow also proposed that this requirement might be labile: although the quantum yield of each rhodopsin molecule, and also its probability per time to isomerize spontaneously, may be fixed, nevertheless the quorum requirement may be adjustable depending on the assigned task. Demanding a low false-positive rate would cause the requirement to be set higher than otherwise, potentially explaining the discrepancy between Hecht's and van der Velden's results [8].

Sakitt picked up this thread with an elegant psychophysical experiment. Instead of a binary seen/not-seen report, she asked each subject to *rate* each flash presented on a scale from $r = 0$ ('did not see anything') to $r = 6$ ('very bright flash'). In fact, each 'flash' was randomly chosen by the experimenter from among three possibilities, with mean count equal to 0 (no flash, or 'blank'), 55, or 66 photons delivered to the eye. Figure 3 shows that, as might be expected, blanks mostly elicited reports of 0 but occasionally something higher. As the true flash strength increased, the distribution of reported rankings moved to the right.

Following Barlow's hypothesis, Sakitt pointed out that the actual number of photon-like rod signals in an integration time (around 200 ms [10 figure 7.5]) around the putative flash time, and in a summation region about the expected flash location, is a Poisson-distributed variable, whose mean is the sum of two contributions. The first contribution, $\mu_{0,\text{spot}}$, is the mean rate of spontaneous signals from one rod, times the number of rod cells in the summation region, times the integration time.

The second contribution is the actual number of photons presented in the flash, $\mu_{\text{ph,spot}}$, reduced by a multiplicative factor (the retina's 'quantum catch' Q_{spot}). The quantum catch is the fraction of incident photons that get productively absorbed and trigger a signal, that is, those not lost to reflection from the cornea, absorption en route to the retina, or other non-productive absorptions.

All together, then

> The number of photon-like rod signals in an integration time and a summation region is a Poisson random variable with expectation $\mu_{\text{tot}} = \mu_{0,\text{spot}} + Q_{\text{spot}}\mu_{\text{ph,spot}}$. (1)

In this formula, $\mu_{0,\text{spot}}$ and Q_{spot} are constants for any subject; $\mu_{\text{ph,spot}}$ is under experimental control. Sakitt then assumed that

(a) some neural mechanism computes the quantity μ_{tot}, that is, sums the contributions from rod cells;

(b) some neural mechanism deterministically compares μ_{tot} to each of several quorum values

[2] The quorum requirement is often called a 'threshold,' but in this article that word is reserved for the threshold at the first synapse, which is applied to a continuous quantity (change in glutamate concentration). To avoid confusion, the phrase 'quorum requirement' will be used for minima applied to integer quantities (photon absorptions in a single rod, or rod signals in a summation region).

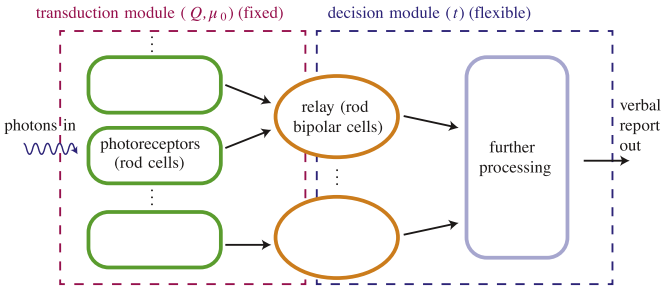

Figure 4. Logical structure of the model used in this article. A 'transduction module' applies a Bernoulli trial to each incoming photon with probability of productive capture Q; it also generates additional isomerization events in the dark at rate μ_0. A subsequent 'decision module' then sums the resulting signals and applies one or more quorum requirements t. *Rounded boxes* symbolize actual wetware in the eye. Rod bipolar cells straddle the logical processing elements because they do some thresholding at the first synapse (section 5) as well as some summation.

$t_1, \ldots, t_6$ to decide whether the requirements for each rating have been met;

(c) the human subject responds to each trial with the name of the highest rating whose requirement was met;

(d) the subject has previously set the quorum values during training.

Notice that the model embodied by these points assumes that neural processing is *perfect*, that is, it introduces no loss of signals nor randomness of response. The only sources of loss and randomness are assumed to be the unavoidable, physical (and as we will see measurable) ones implicit in equation (1). In particular, figure 3 shows some nonzero responses to blanks; the model assumes that these were the result of a deterministic neural processing of an event in which the number of spontaneous isomerizations exceeded t_1.

In the following decades, much was discovered that made these assumptions seem naive. For example, we now know that rod signals fan out, then recombine at various stages on their journey to the visual cortex. Additional noise can arise at the rod bipolar to AII amacrine synapse and elsewhere in the AII network [11], for example, due to the discreteness of neurotransmitter vesicle release. Signals also can be lost at every stage. Nevertheless, section 6.3 below will explore an updated version of Sakitt's model because it is simple and concrete. Also, the fact that, as we will see, it can reproduce complex experimental data in detail places severe constraints on future models we may entertain for the actual neural processing used in dim-light vision. The update consists of incorporating some single-cell data, reviewed in the following sections. Figure 4 sketches the logical structure of the model's assumptions.

4. Single-cell measurements

4.1. Baylor *et al*

Once again, we may be tempted to express Sakitt's model in mathematical terms and attempt to fit it to her data. The usefulness of that program is limited, however, by the fact that many different fits are possible [12]. For example, psychophysics alone cannot tell us the retina's quantum catch: perhaps it is low, but also the false-positive rate is low as well. Then we can set low quorum requirements and still achieve the level of reliability seen in the data. Alternatively, the quantum catch may be high, but the false-positive rate is also high; then we can get a similar fit by setting high quorum requirements.

We can do better than this if we augment the psychophysical experiments with single-photoreceptor physiology. Yau, Lamb, and Baylor overcame the daunting technical obstacles a few years after Sakitt's experiment [13]. They were able to isolate individual photoreceptor cells, interrupt their extracellular current path, and in that way record changes in the current elicited by dim flashes of light. Later work found clear signals in which the extracellular current briefly dropped by about a picoampere in response to weak flashes of light, a response well distinguished from lower-amplitude background ('continuous noise').

Imagining each rod photoreceptor cell's outer segment as a cylindrical tube packed with rhodopsin molecules, we can expect that each cell will have a single-cell quantum catch Q_{rod}, analogous to the whole-retina quantum catch mentioned earlier. Suppose that we present flashes of light that deliver an average of $\mu_{\text{ph,rod}}$ photons to the rod cell's outer segment. Then

> The number of productive absorptions, ℓ, elicited by such flashes will be a Poisson random variable with expectation given by $\langle \ell \rangle = Q_{\text{rod}} \mu_{\text{ph,rod}}$. (2)

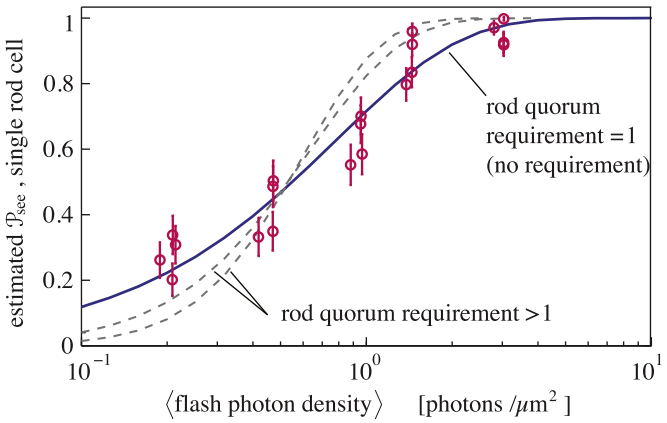

Figure 5. Determination of rod quorum requirement. Circles: probability that a single rod cell will respond to a dim flash of 500 nm light, for four different individual cells from macaque. Later experiments confirmed that human rod cells behave similarly. The rod outer segment was illuminated from the side; the logarithmic horizontal axis gives the mean number of photons presented divided by the sideways cross sectional area of the cell (about 50 μm^2). The error bars represent one standard deviation, based on the variance of a Bernoulli trial repeated 65 times. The solid curve shows a fit to a physical model that assumes that the productive absorption of a single photon can give rise to a signal; dashed curves show attempted fits to alternative models, in which two or three photons must be simultaneously absorbed. Data from [14].

(In principle, the mean number of spontaneous isomerizations should be added to this expression, but at the single-rod level there is no pooling among many rods, so the probability for such an event to coincide with a stimulus flash may be neglected.)

Baylor and coauthors entertained various hypotheses about the relation between ℓ and the photoreceptor's output:

H1: The photoreceptor may be able to respond reliably any time $\ell \geqslant 1$.

H2: The photoreceptor may respond reliably only when ℓ exceeds some quorum requirement, such as $\ell \geqslant 2$, and so on.

To choose between these hypotheses, they made a *single-cell* 'probability of seeing' graph, showing the probability of a rod cell responding as a function of the intensity of the stimulus. Figure 5 shows such graphs for four cells on semilogarithmic axes. Hypothesis H1 and equation (2) predict that

$$\begin{aligned}\mathcal{P}_{see}(\mu_{ph,rod}) &= 1 - \exp(-Q_{rod}\mu_{ph,rod}) \\ &= 1 - \exp(-\exp(\ln Q_{rod} + \ln \mu_{ph,rod})). \qquad \text{H1}\end{aligned}$$

The last expression may seem to be a perverse way of writing something simple, but it emphasizes a key point: changing the value of Q_{rod} just pushes the semilog graph of the predicted $\mathcal{P}_{see}$ horizontally, without altering its shape. Thus, if the hypothesis makes the wrong prediction for the maximal slope of the data, *changing the fit parameter* Q_{rod} *will not help,* and similarly for H2.

The figure shows that hypothesis H1 can be fit to describe the data, but H2 and higher cannot. In this way, Baylor and coauthors vindicated Hecht and coauthors' conclusion that *individual rod cells impose no quorum requirement* on their primary measurement, which is the number of rhodopsin molecule isomerizations that take place in an integration time. (Of course, it is still possible that later stages of processing impose such requirements, as Barlow proposed.)

In addition to rejecting some hypotheses, data like those in the figure allowed Baylor and coauthors to determine the quantum catch for single rod cells for the animal (macaque) and the illumination method (sideways) that they used: $Q_{rod} = 0.025$ gives the fit shown in the figure.

By turning off the illumination, Baylor and coauthors were also able to determine the mean rate of false positive signaling: a more modern measurement gives a value similar to theirs, again for macaque: 0.0037 S^{-1} (Greg Field, personal communication).

To summarize, single-cell physiology gave a more nuanced version of the claim that our visual apparatus can 'respond to single photons':

> When presented with a burst of photons directed perpendicular to its axis, a photoreceptor cell responds to each photon independently, either signaling (with probability Q_{rod}) or not (probability $1 - Q_{rod}$). (3)

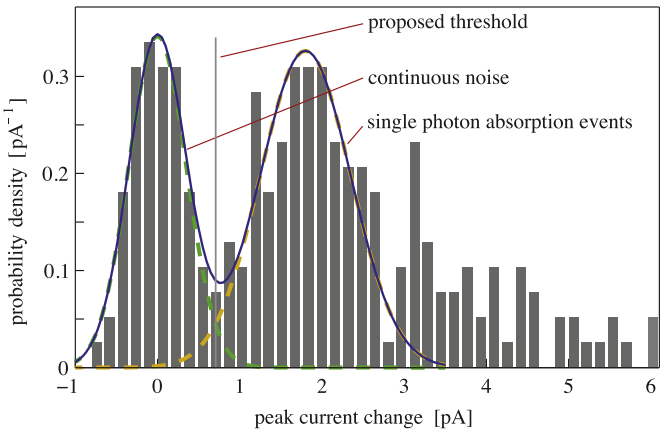

Figure 6. The distribution of false positive signals overlaps that of true positives. Bars: observed distribution of peak amplitudes of a rod cell's extracellular current change after a flash of light. Solid curve: an idealized probability density function, the sum of two Gaussian distributions corresponding respectively to continuous noise and events in which a single productive photon absorption occurred (dashed curves). We might declare that any event exceeding the value marked by a vertical line will be deemed an isomerization event, but the graph suggests that a fraction of those events really belong to the continuous-noise peak. Data courtesy Greg Field; see also [12].

4.2. Direct determination of rod cell quorum requirement

Section 4.1 discussed a classic demonstration that rod cells impose no quorum requirement: they can signal after productively absorbing a single photon. These experiments relied on indirect, probabilistic reasoning, however, because light sources available at the time could not produce flashes containing exactly one photon. One could reduce the flash intensity or duration to the point where the flashes have mean number of photons μ_{ph} smaller than one. Then, each 'flash' is unlikely to contain more than one photon; but most contain none at all. When such stimuli are presented to an isolated rod cell, it does sometimes respond, but this does not directly prove that the rod can respond to single productive absorptions—instead, it is possible that some of the observed rod signals were spontaneous, false-positive events that would have happened with *no* photons. Comparing the rod signals with and without the flashes is difficult, because a small difference of two noisy quantities has high relative standard deviation.

More recent techniques, however, do allow the creation of one-photon states. Phan and coauthors used one such method to revisit the question of a possible rod quorum requirement [15]. To overcome the difficulty of many zero-photon states, Phan and coauthors passed their light flashes through a crystal of β-barium borate. The crystal let most photons pass through unchanged, but converted a small fraction into *pairs* of photons. Each photon in the pair emerged at the same time and had the same wavelength, which was chosen to match the rod sensitivity peak. One of the pair was directed to a rod cell in a suction pipette apparatus. The other was directed to a sensitive detector. Signals from this detector were reliable indicators of when the rod cell received exactly one photon. The experiment found that on a significant fraction of trials the rod cell responded to single photons, giving direct evidence that it imposes no quorum requirement.

5. The first synapse

The previous section characterized rod cell responses to dim flashes: they effectively subject each photon to a Bernoulli trial and count the resulting productive absorptions. Following Barlow and Sakitt, we could now attempt to model everything else in the visual system as deterministically summing and applying thresholds to the number of rod signals. However, more recent experiments allow us to introduce one more realistic element: transmission through the synapse from rod to rod bipolar cells. Our strategy will be to characterize this processing step experimentally, then idealize everything *else* as a sum/threshold operation.

Figure 6 shows that there is a fairly clear demarcation between the weaker continuous rod noise and the larger current drops generated by photon absorptions (plus occasional spontaneous isomerizations)[3]. If we were given such a signal, we might interpret it by just declaring that every event with peak current change less than, say, 0.8 pA is continuous noise, and the rest represent isomerization events. But this strategy would not give us good vision for the following reason.

Although the signals from each individual rod cell seem to split nicely into signal and noise, nevertheless figure 6 shows some overlap between the two distributions. The thousand or so rod cells whose outputs are pooled to give the signal corresponding to a particular

[3] Continuous noise is thought to be at least partly due to spontaneous activation of phosphodiesterase.

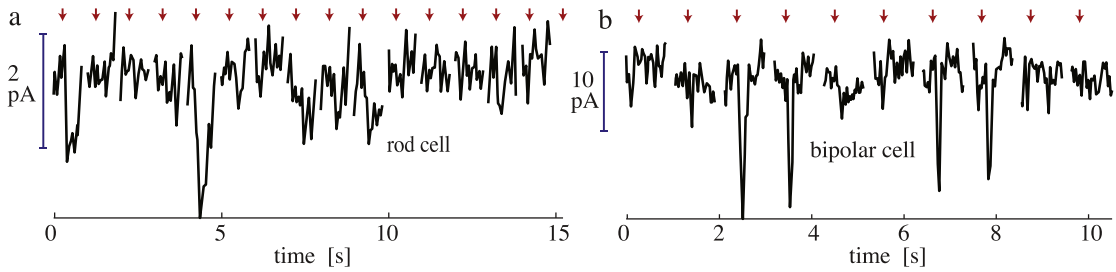

Figure 7. Effect of thresholding at the first synapse. (a) Responses to light flashes by single mouse rod cells. Circulating current is shown as a function of time. Light flashes were delivered at times marked by arrows. (b) Responses recorded from mouse rod bipolar cells. Thresholding at the first synapse has made the true signals stand out from noise better than in (a). Data from [16].

visual region are all constantly making this noise, even in darkness. If our eyes really set a threshold like the one imagined in figure 6, then we'd misclassify a small but significant fraction of the noise events as signals, totally swamping the genuine signals, which are rare in dim light.

Baylor, Nunn, and Schnapf pointed out this difficulty, and offered a possible resolution. They reasoned that *noise could be suppressed by sacrificing some real rod signals.* Suppose that we select the criterion 1.8 pA, considerably higher than the 'obvious' breakpoint in figure 6. Then about half of the isomerization signals (second peak in figure 6) will be wrongly discarded. The payoff, however, is that then practically *none* of the noise signals (first peak) will be wrongly passed on to the next neuron (retinal bipolar cell)—a big improvement to the net signal to noise ratio, at the cost of reducing the overall quantum catch [3].

Baylor and coauthors noted that this thresholding operation must take place downstream from the rod cell, because continuous noise arises in the rod. It must also take place before any pooling of signals is done, because after many rod signals have been combined, it is too late to determine that some of them were noise and should be deleted. Because about 15–30 rod cells all combine their signals as inputs to the next level of processing (the rod bipolar cell), this logic implies that the thresholding must occur at the synapse between a rod and its bipolar cell. Indeed, direct evidence for this hypothesis came years later, when techniques became available to record from the bipolar cells (see figure 7).

The origin of thresholding lies in the fact that in the dark, more than enough glutamate is released by a rod cell to keep every mGluR6 receptor on the other side of the first synapse saturated, so the corresponding ion channels that they control remain closed. A small hyperpolarization of the rod, leading to a small reduction of glutamate release, does not allow any of the channels to open. Once a threshold is reached, however, the high cooperativity of receptor activation creates a significant signal [17].

How high is this synaptic threshold? Berntson and coauthors noted that thresholding can do nothing about the noise generated by spontaneous isomerizations of rhodopsin, because those events look *exactly* like the ones actually elicited by photons. So there is little point in raising the synaptic threshold indefinitely: once the rate of false positives from the continuous noise (no isomerizations) has been reduced to less than that from spontaneous isomerizations, increasing the threshold further would cut the quantum catch without much improvement in the signal/noise ratio. These researchers estimated that, in mammals, the tradeoff point comes when about 50% of the true photon events are sacrificed [11, 17].

6. The modeling challenge

Sections 2 and 3 outlined experiments on human subjects, but pointed out that too wide a range of mechanistic models can be fit to such data. Sections 4 and 5 outlined experiments on individual cells, the inputs to further neural processing. It is natural to ask whether we can accommodate both classes of experiments in a single model similar to Sakitt's. To do this, we must somehow translate the single-cell parameters, the quantum catch Q_{rod} and spontaneous signaling rate (for macaque), into estimates of the parameters Q_{spot} and $\mu_{0,\text{spot}}$ appearing in equation (1) (for humans). The following sections carry out this translation, including the effects of thresholding at the first synapse. We must then check whether there exists any choice of quorum values t_i that fit the data in a Sakitt-type experiment, and if so, interpret the result.

Sakitt's model is highly falsifiable, because the only remaining fitting parameters are constrained to be a few small integers (the t_i), and because the data to be fit are a function of two variables: $\mathcal{P}_{\text{see}}(\text{ rating } | \text{ stimulus})$ (see figure 3). Moreover, the physics of light and of photoisomerization bounds the loss and randomness of the system from below (the photons in a flash are Poisson-distributed, and each photon makes a random choice whether to be productively absorbed), whereas the extraordinary performance of the overall system bounds the loss and randomness from *above*. Is

there any room between those bounds, and if so, how much?

6.1. From sideways to axial illumination

Screening Baylor and coauthors subjected single rod cells to light flashes of various strengths, obtaining 'probability of seeing' data such as those in figure 5. However, their experiment exposed the rod to light directed *sideways* (transverse to its axis). We'd like to deduce the quantum catch of a rod for light that passes through it *axially*, as it does in an intact eye. To make the connection, we must consider how an optically dense medium absorbs light[4].

The Beer-Lambert formula gives the probability that an incident photon will be transmitted by a thick absorbing sample as

$$\mathcal{P}(\text{ transmitted}) = \exp(-\sigma c X). \tag{4}$$

Here X is the sample thickness, σ is the absorption cross-section, and c is the number density (concentration) of molecules that absorb light in the frequency range of interest. (Some authors rewrite this formula as $10^{-\zeta X}$, where the absorption coefficient is defined as $\zeta = \sigma c/(\ln 10)$.) If $\sigma c X \ll 1$, then we may approximate the probability that the photon *will* be absorbed as

$$\mathcal{P}(\text{ absorbed}) = 1 - \mathrm{e}^{-\sigma c X} \approx \sigma c X. \quad \text{(thin sample approximation).} \tag{5}$$

Baylor and coauthors used rod cells from macaque monkeys, which have outer segment diameter $d_{\text{rod}} = 2\ \mu$m and length $L^{\text{m}}_{\text{rod}} = 25\ \mu$m [14]. For rhodopsin at the concentration found in rod cells, the measured value of σc is $0.044\ \mu\text{m}^{-1}$ [18]. Thus, for sideways illumination of rod cells, equation (5) is a good approximation, because any sideways path through the rod has length less than or equal to d_{rod}, and $d_{\text{rod}}\sigma c = 0.09 \ll 1$. In the experiments the light beam had uniform intensity over a rectangle of size $d_{\text{rod}} \times L_{\text{rod}}$ (just covering the outer segment). Averaging the absorption probability over the variable thickness of a cylinder across its cross section gives

$$\mathcal{P}(\text{ absorbed (sideways)}) = \sigma c\,(\pi d_{\text{rod}}/4). \tag{6}$$

The quantum yield for rod signaling. We can now find the probability ϕ_{sig} that an absorbed photon actually triggers a rod response, by combining equation (6) with the definition of conditional probability:

$$\phi_{\text{sig}} = \mathcal{P}(\text{rod signal} \mid \text{absorbed (sideways)}), \tag{7}$$

$$= \mathcal{P}(\text{rod signal } \textbf{and} \text{ absorbed (sideways)})/\mathcal{P}(\text{ absorbed (sideways)}). \tag{8}$$

The numerator of this expression is the rod quantum catch (section 4.1), so we find

$$\phi_{\text{sig}} = \frac{Q_{\text{rod}}}{\sigma c \pi d_{\text{rod}}/4}. \tag{9}$$

We will call ϕ_{sig} the 'quantum yield for signaling' in primate rod cells.

Section 4.1 gave $Q_{\text{rod}} = 0.025$, so substituting other given numbers yields

$$\phi_{\text{sig}} = \frac{0.025}{0.044\ \mu\text{m}^{-1} \cdot \pi \cdot 2\mu\text{m}/4} = 0.36. \tag{10}$$

For present purposes, the interest of ϕ_{sig} lies in the fact that its value should be *the same regardless of how light has been presented* to the rod cell. Once a photon has been absorbed by rhodopsin, its original direction no longer matters:

$$\mathcal{P}(\text{rod signal} \mid \text{absorbed (axial)}) = \mathcal{P}(\text{rod signal} \mid \text{absorbed (sideways)}) = \phi_{\text{sig}}.$$

We now wish to use this fact to find the rod's quantum catch for *axial* illumination—the case of interest for psychophysics.

Quantum catch for a single rod cell under axial illumination. To estimate $Q_{\text{rod,axial}}$, we start with the probability for a photon to be absorbed when presented axially to a human rod, whose outer segment length at 11° temporal eccentricity is $L^{\text{h}}_{\text{rod}} \approx 42\ \mu$m [19], then multiply it by ϕ_{sig} (see equation (10)):

$$\begin{aligned} Q_{\text{rod,axial}} &= \mathcal{P}(\text{rod signal (axial)}) \\ &= \mathcal{P}(\text{rod signal} \mid \text{absorbed (axial)}) \\ &\quad \times \mathcal{P}(\text{absorbed (axial)}) \\ &= \phi_{\text{sig}}\,(1 - \exp(-\sigma c L^{\text{h}}_{\text{rod}})). \end{aligned} \tag{11}$$

Note that the thin-sample approximation equation (5) is *not* valid. We might have expected that: for good night vision, rod cells need to catch a large fraction of the photons that arrive on them, so $\exp(-\sigma c L^{\text{h}}_{\text{rod}})$ should not be close to 1. Substituting numbers given earlier yields $Q_{\text{rod,axial}} \approx 0.30$.

Absorption by ocular media. We have now obtained a numerical value for the quantum catch of one rod. Before we can interpret behavioral experiments, however, we need the probability Q_{spot} that the *entire* retina will respond to an incident photon. Photons must pass several hurdles before they arrive at a rod cell; each hurdle multiplies the probability of productive absorption by a number smaller than 1. All of these factors vary considerably between individuals, so any calculations we make with them will be approximate.

The first factor arises because about 4% of the incident light is reflected from the cornea—it never enters the eye at all. There is also loss due to absorption and scattering in the cornea, eye lens, and fluids prior to the retina. This quantity is dependent on the subject's

[4] Baylor and coauthors took care to apply light with a polarization that lay in the plane of the disk membranes. Axially directed light always has such a polarization, but sideways illumination can have a component perpendicular to the disks and hence to the retinal chromophore's transition dipole. Eliminating the perpendicular component ensured that the sideways illumination could be meaningfully compared to the natural (axial) situation.

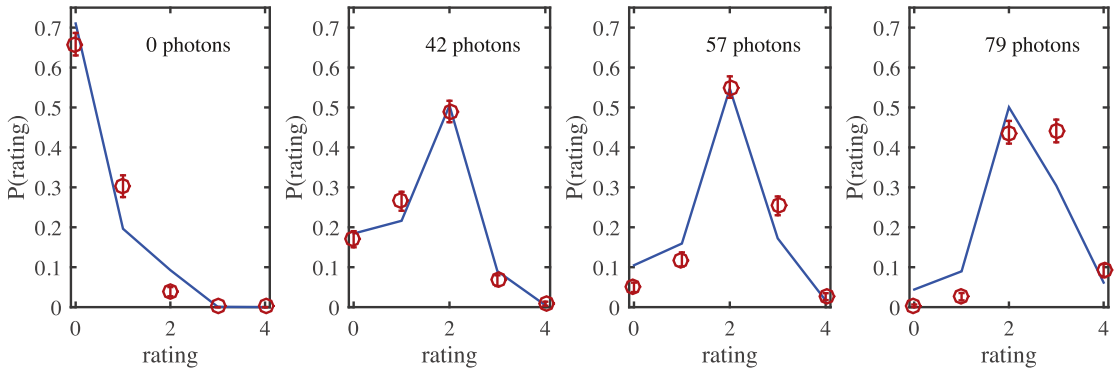

Figure 8. Fit to a modern version of Sakitt's experiment. Circles show the estimated $\mathcal{P}$(rating; stimulus), that is, the observed probabilities of various ratings given by one subject, for flashes of 490 nm light with four different strengths. Lines show a maximum-likelihood fit to the model developed in the text, with quantum catch and noise parameters fixed by single-cell measurements. Thus, the only fitting parameters are the assumed quorum requirements for each of the four ratings. Error bars indicate one standard deviation for a Poisson distribution with expectation equal to the number of times each rating was given. Data courtesy Darren Koenig and Heidi Hofer; see also [23].

age; a typical value, appropriate for the 22 year-old subject whose performance is shown in figure 8, viewing light of wavelength 490 nm, is 0.47 ([20 equation (8)]). Multiplying by 0.96 for corneal reflection gives the 'ocular media factor':

$$[\text{ocular media}] \approx 0.45.$$

Tiling factor. Next, we must account for the fact that rods do not completely fill the retina's surface, so some of the photons that arrive at the retina do not enter any rod. This 'tiling factor' depends on where on the retina we choose to direct the flash. K. Donner estimated it as 75% at 17° temporal eccentricity ([21 table 1]). For the experiment to be analyzed in section 6.3, we must correct this factor for the slightly different area density of rod cells at 11° temporal eccentricity, a factor of $1.3 \times 10^5\,\text{mm}^{-2}/1.4 \times 10^5\,\text{mm}^{-2}$ [22]:

$$[\text{tiling}] \approx 0.70.$$

Thresholding at the first synapse. A third loss mechanism, introduced in section 5, discards some true (and false) photon signals. Section 5 quoted an estimate of this 'synaptic threshold loss factor' as

$$[\text{thresh}] \approx 0.5.$$

We can now combine these factors to estimate the effective quantum catch for the human eye, that is, the fraction of photons incident on the eye that actually excite signals in the rod bipolar cells:

$$Q_{\text{spot}} = [\text{ocular media}] \times [\text{tiling}] \times [\text{thresh}] \times Q_{\text{rod,axial}}. \quad (12)$$

Evaluating this expression gives $Q_{\text{spot}} \approx 0.048$.

6.2. Spontaneous signaling

The volume of a human rod cell's outer segment at 11° temporal eccentricity is about 1.68 times that of the macaque cells studied by Baylor *et al* [14, 19, 21]. So we expect the total number of rhodopsin molecules, and hence the rate of spontaneous isomerizations, also to scale by this factor: mean rate $\approx 0.0062\ \text{s}^{-1}$ (see section 4.1). Thus, the number of spontaneous events that could be confused with real photon absorptions is roughly this rate, multiplied by the rod integration time, about 200 ms, and reduced by 50% to account for loss at the first synapse. (We ignore the contribution to the false-positive rate from continuous dark noise, because as we have seen, the first synapse imposes a threshold that eliminates it.) The number of rod cells signals that are effectively pooled is not well measured, but Barlow found a value equivalent to about 1700 [10], which leads to the estimate

$$\mu_{0,\text{spot}} \approx (0.0062\ \text{s}^{-1}) \times (200\ \text{ms}) \times 0.5 \times 1700 \approx 1.06. \quad (13)$$

6.3. Another recent experiment

Koenig and Hofer recently performed a modern version of Sakitt's experiment, but with fewer allowed ratings (hence fewer fit parameters) and more flash strengths (hence greater falsifiability). Figure 8 shows data for one of their subjects[5]. We wish to fit such data with a Sakitt-type model, updated to include the effect of signal loss at the first synapse.

The model states that every 'flash' (including nulls) generates a Poisson-distributed number of photon signals, ℓ, with mean given by equations (1), (12) and (13). Thus, for each flash strength the model predicts the full distribution of ℓ. Starting from a set of integers $t_1, \ldots t_4$, we then obtain the probability that the flash elicits rank 0 as the sum of the probabilities for $\ell = 0, \ldots, t_1 - 1$, and so on. In this way, we construct the model's predicted probability distribution $\mathcal{P}$(rating | stimulus). We use these distributions and

[5] Koenig and Hofer studied four human subjects in this part of their experiment. The one chosen for analysis here was given conditions in which the weak-intensity flashes had the smallest numbers of photons, and hence probed threshold vision most adequately.

the actual experimental data to generate a likelihood function, and optimize it over various sets of increasing integers $\{t_i\}$. We do *not* fit the parameters Q_{spot} nor $\mu_{0,\text{spot}}$. Compared to other analysis methods such as receiver operating characteristic curves [24], this one appears more directly related to the raw experimental data (frequency of seeing).

Figure 8 shows the best fit found in this way for one subject, using the values $t_r = 2, 3, 6$, and 9 for the quorum requirements associated to the four allowed ratings. Other subjects gave similar fits, generally with t_1 between 2 and 3. We conclude that a Sakitt-type model can account for psychophysical performance, even when constrained by single-cell physiology, and that the passage of as few as two signals through the first synapse is enough to generate a measurable behavioral response.

6.4. Uncertainties in parameter values

All the parameter values used in this analysis are subject to normal variation between subjects, but the rod outer segment length has a particularly broad range of literature values (25–50 μm). The fit in figure 8 used a value specifically measured at the retinal position relevant to the psychophysical experiment in [23]; other positions have different lengths, possibly accounting for the wide range of quoted values [19]. Nevertheless, it is interesting to see how sensitive the fit is to the value of this parameter.

Using 25 μm in the analysis gave a best fit that was technically worse (lower likelihood) than the one shown in figure 8, with thresholds $t_r = 1, 2, 4$, and 7. Using the other extreme value, 50 μm, actually gave a technically better fit, again with $t_r = 2, 3, 6$, and 9.

7. What has/has not been shown

Returning to the questions in section 1, we now see that (a) is not very well posed: the number of photons in a flash is not even under our experimental control (unless we use exotic light sources as in section 4.2). However, we can say that the dim-light visual system, in a dark-adapted human subject, does behave as though *two* signals passing the first synapse in an integration time, within a summation region, were enough to bias the conscious mind toward perceiving a flash, and around ten suffice to give certainty.

Concerning question (b), some loss is inherent in the media constituting our eyelens and other components. Some comes from the finite absorption cross-section of rhodopsin and the finite length of rod cells. Some loss comes from a tradeoff at the first synapse, involving the continuous noise generated by rod cells and the pooling of signals needed at low light intensity. Finally, light flashes eliciting fewer than about ten signals crossing the first synapse generate limited conviction in an observer because of the spontaneous thermal isomerization inherent in rhodopsin's molecular design.

Along the way to these conclusions, the analysis addressed question (c) by using an appropriate probabilistic model.

A fourth question was asked in section 6: now that we have found *one* model that reconciles psychophysics and single-cell physiology, are there others? How much room is there between the lower bound on loss and randomness found at the single cell level, and the upper bound imposed by the whole-animal performance? To answer such questions, we would need a broader class of models, rooted in more realistic assumptions about the processing that occurs after the first synapse. Existing psychophysical data are not extensive enough to address this question systematically, nor are existing estimates on some of our model's parameters very precise (for example, the size of the summation region). However, if we model additional signal loss as a reduction of the effective quantum catch below the value used above, then the best fit to data is much worse with even a 20% reduction. Similarly, if we represent additional randomness by a larger effective value of the false signaling rate $\mu_{0,\text{spot}}$, we find no fit is possible if $\mu_{0,\text{spot}}$ is taken to be 30% larger than the estimate made above. So we can say in broad terms that neural processing after the first synapse is highly efficient; most of the loss and randomness that set our dim-light visual performance stem have physical origins, and are already accounted for once signals have passed the first synapse.

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