New light on photon detection
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A human is capable of detecting a flash of light delivering just five photons to the retina. This astounding feat has profound implications for understanding how signals are generated and transmitted in the visual system. To detect very dim light, absorption of a photon by a rod photoreceptor must generate a response that is reliably transmitted across the synapse to second-order bipolar cells. The use of suction electrodes to record the current flowing across the rod outer segment has provided beautiful measurements of this single photon response, but it has also demonstrated that these signals are relatively small and contaminated by significant amounts of noise originating in the phototransduction mechanism (Baylor et al. 1979). Further ‘synaptic’ noise will be added to the output from the rod because neurotransmitter is released probabilistically, in quanta corresponding to single vesicles. How is the absorption of a photon detected above these two sources of noise?

To answer this question, our first task is to quantify the size of the single photon event in the rod relative to the background noise – the signal-to-noise ratio (SNR). It has generally been thought that absorption of a photon hyperpolarizes a mammalian rod by a little less than 1 mV, with a SNR of 3 (Schneeweis & Schnapf, 1995; Field & Rieke, 2002). This SNR is relatively low, and its implications are shown in Fig. 1: counting a true photon absorption would be difficult because there is significant overlap in the distributions of noise values and the amplitudes of single photon events. Field & Rieke (2002) suggested that the decision to count a photon arises at the synapse from the rod to the bipolar cell through application of a threshold. To understand how this threshold might operate, it is important to understand that rods hyperpolarize to light, so that the signal transmitted is a decrease in release of glutamate. They proposed that fluctuations in the rod voltage below a threshold are not transmitted because post-synaptic glutamate receptors are saturated (thereby rejecting a large fraction of the noise), while crossings of the threshold are counted as photon arrivals.

At what level is this threshold set? This question can be probed by repeatedly applying very dim flashes, which on average cause significantly less than one photon to be absorbed in the rod: variations in the response will then follow Poisson statistics, with the variance equal to the mean. But a threshold at the rod synapse that causes a proportion of events to be ‘lost’ will increase the variance of the response in the bipolar cell above the Poisson prediction.

Figure 1. Detecting a photon above noise
A, top, the response of a rod to a single photon in the absence of noise. Below are shown simulated responses in the presence of noise with a SNR of 3 (black) and 6.5 (red). The two responses are normalized to the amplitude of the noise, which has a standard deviation of 0.33. Thus when the SNR is 3, the mean response to a single photon is 1. The horizontal green line shows a threshold for photon detection of 1.3. B, distribution of responses to repeated trials in which half do not lead to photon absorption (the peak centred on zero) and half lead to absorption of one photon. Note that the response to a single photon is itself variable, with a standard deviation of 0.33 times the mean. With a SNR of 3, a relative threshold of 1.3 would reject almost all the noise and prevent detection of false positives (dotted trace), but the majority of single photon events would also be lost (thinner solid trace). The same threshold operating on responses with a SNR of 6.5 would detect a much larger fraction of absorbed photons.
Careful modelling of this situation has led to different views. One study suggests that the synaptic threshold would be about 1.3 times the average amplitude of the single photon event to account for the statistics of the response in the bipolar cell when the SNR in the rod is $\sim 3$ (Field & Rieke, 2002). This is a surprisingly high value, because it would cause $\sim 75\%$ of single photon absorptions in the rod to be lost. In contrast, Berntson et al. (2004) found that the dim flash response in mouse rod bipolar cells did not deviate from Poisson statistics, suggesting that the synaptic threshold was smaller than the average amplitude of the single photon response to allow a larger fraction of photon absorptions to be transmitted.

These calculations depend critically on good measurements of the SNR of the single photon event in a mouse rod. But rather than measure the current flowing across the outer segment, one would like to measure the voltage in the inner segment because this is the signal that controls neurotransmitter release from the terminal. Such voltage recordings have proven extremely difficult to make in mammalian rods, and the only measurements we have had so far come from a series of beautiful studies in macaque by the group of Julie Schnapf (Schneeweis & Schnapf, 1995). But now, in an article in this issue of *The Journal of Physiology*, Cangiano et al. (2012) have honed their technique to obtain voltage recordings from rods and cones in slices of mouse retina.

A key finding of the study of Cangiano and colleagues is that the single photon response is much larger than previously thought, about 2.5 mV with a SNR of $\sim 6.5$. This finding has immediate implications for understanding the operation of the rod synapse: in the dark, the threshold for transmission could be set low to cut-out noise while allowing transmission of the single photon event (Fig. 1). A scenario in which the single photon event is large enough to be distinguished from noise with a low threshold makes intuitive sense because fewer precious photons will be lost (Berntson et al. 2004).

The work of Cangiano and colleagues (Cangiano et al. 2012) will have profound implications on future experimental and theoretical work seeking to understand how the retina detects the dimmest lights.

**References**

Baylor DA, Lamb TD & Yau KW (1979). *J Physiol* **288**, 613–634.

Berntson A, Smith RG & Taylor WR (2004). *Vis Neurosci* **21**, 693–702.

Cangiano L, Asteriti S, Cervetto L & Gargini C (2012). *J Physiol* **590**, 3841–3855, May 28. [Epub ahead of print].

Field GD & Rieke F (2002). *Neuron* **34**, 773–785.

Schneeweis DM & Schnapf JL (1995). *Science* **268**, 1053–1056.