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Low retinal noise in animals with low body temperature allows high visual sensitivity

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The weakest pulse of light a human can detect sends about 100 photons through the pupil and produces 10–20 rhodopsin isomerizations in a small retinal area[1,2]. It has been postulated[3] that we cannot see single photons because of a retinal noise arising from randomly occurring thermal isomerizations. Direct recordings have since demonstrated the existence of electrical 'dark' rod events indistinguishable from photoisomerization signals[4–6]. Their mean rate of occurrence is roughly consistent with the 'dark light' in psychophysical threshold experiments, and their thermal parameters justify an identification with thermal isomerizations[5]. In the retina of amphibians, a small proportion of sensitive ganglion cells have a performance-limiting noise that is low enough to be well accounted for by these events[7–10]. Here we study the performance of dark-adapted toads and frogs and show that the performance limit of visually guided behaviour is also set by thermal isomerizations. As visual sensitivity limited by thermal events should rise when the temperature falls, poikilothermous vertebrates living at low temperatures should then reach light sensitivities unattainable by mammals and birds with optical factors equal. Comparison of different species at different temperatures shows a correlation between absolute threshold intensities and estimated thermal isomerization rates in the retina.

For the study of factors limiting the absolute sensitivity of vision, the toad *Bufo bufo* offers decisive advantages. First, it is a close relative of *Bufo marinus*, which has provided the best understanding of 'dark' rod events of any species[5]. Second, it is known to manage visually guided prey-catching at light levels so low (<10 μlux) that a human observer cannot see the toads or their prey[11]. Third, both the behavioural threshold of the whole animal and electrophysiological thresholds for retinal ganglion cell responses can be determined under equivalent conditions. We determined both kinds of thresholds at 15 °C and under conditions where stimulus intensities in the retina could be accurately calculated in terms of isomerizations per rhodopsin molecule per second (denoted R^*s^{-1}).

For a behavioural experiment, a fully dark-adapted toad in a plastic box was illuminated from above by a green light (525 nm) of adjustable intensity (Fig. 1). Underneath the transparent floor, white 'worm-dummies' (3 × 20 mm) moved over a black background. The dummies appeared at 12 s intervals, but the toad could not see more than one at a time. The occurrence of one or several snaps (acoustically recorded and taped) against the bottom of the plastic box was regarded as a positive response.

The results are summarized in Fig. 2*a* and *b*. The common abscissa gives stimulus intensity, that is, the isomerization rate produced by the dummy, and the black arrows mark the rate of thermal isomerizations at this temperature, 4.9×10^{-12} R^*s^{-1}. The relative frequencies of positive snapping responses at various intensities are given by the filled circles in Fig. 2*a*. For comparison, Fig. 2*b* displays thresholds (small dots) and the intensity ranges in which responses could be obtained (horizontal lines) for 18 well characterized retinal ganglion cells in eyecup preparations stimulated with rectangles of green light, equivalent to the retinal image of the dummy in the behavioural experiments. Figure 2*c* exemplifies the response variability of one of the ganglion cells when stimulated ten times with three fixed intensities, one just below and two just above the 50% response threshold.

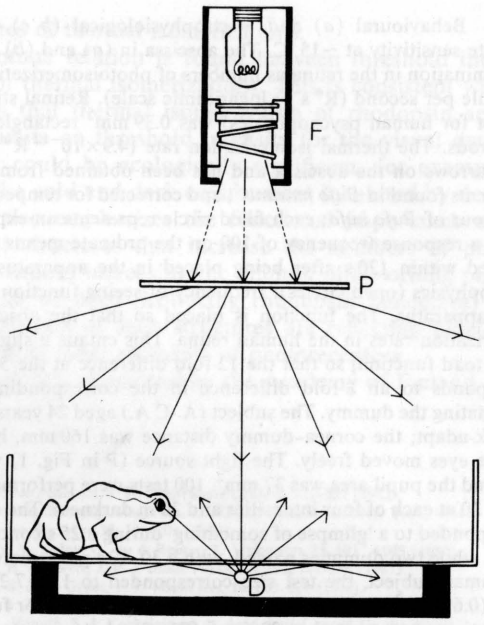

Fig. 1 Schematic presentation of the experimental set-up in the snapping experiments[11]. The apparatus was located in a light-tight black-painted room. The well shielded lamp was connected to a stabilized current source. *F* represents heat-absorbing filters, neutral density filters and the 525 nm interference filter, and *P*, the plastic diffuser screen on which the lamp formed a luminous 39 cm^2 disc 35 cm above the toad. Direct retinal illumination from this 'moon' would be 300 times more intense than that from the dummy, but control experiments in which the source was screened from direct view indicated that its potential visibility had no clear negative effect on snapping sensitivity. *D* is a cross-section through a white worm-dummy (3 × 20 mm, attached to a transparent string and moved at a speed of 8.3 mm s^{-1} and a frequency of one per 12 s). The toad could not see more than one dummy at a time. Optical calculations were checked by direct measurements of reflections, transmissions and absolute intensities using a reflection spectrophotometer[14] and a radiometer calibrated against rhodopsin extracts[8]. The distance between the eye of the toad and the dummy could not be accurately controlled, but was estimated to be 50 mm (ref. 15). Variation in this distance affects the size of the retinal image but not its intensity. For 50 mm distance and 4.2 mm posterior focal length of the eye[16], the image of the dummy occupied 0.39 mm^2 of the retina and 4,500 red rods. The entrance pupil (7.1 ± 0.3 mm^2) was determined by flash photography face-on; 9% of the incoming light was estimated to be reflected from the curved cornea[9]. No corrections were made for possible light losses in the lens, vitreous and neural retina. 34% of 525 nm photons incident on the *Bufo bufo* retina produce isomerizations in red rods (for methods, see ref. 8). 18 carefully selected[11] snapping male toads (*Bufo bufo*, eye diam. 6.0–6.5 mm) from southern Finland were used. They were kept at 12–16 °C, with access to water and dark and moist shelters. All prey-catching experiments were carried out in July and August, noon to 1800 h, at ~15 °C. The toads were always allowed one day of rest between two tests. They were only fed just after each test. Increased reluctance to snap at dummies was observed after two months.

All toads responded promptly in the intensity range from 3×10^{-11} $R^* s^{-1}$ upwards, and the same applied to 14 out of 18 ganglion cells. But between 3×10^{-11} and 3×10^{-12} $R^* s^{-1}$, both ganglion cell and snapping activity decreased sharply. The 50% response threshold of the most sensitive ganglion cell was 3.1×10^{-12} $R^* s^{-1}$, and that was also the lowest intensity for snapping that was obviously light-dependent. One of the four toads responding at that intensity did snap once even at 6×10^{-13} $R^* s^{-1}$, and also snapped once in complete darkness. As no other toad ever responded at lower intensities, the light-dependence of the snapping at 3.1×10^{-12} $R^* s^{-1}$ (4 toads snapping out of 18 tested) is statistically significant at $P < 0.01$.

It appears remarkable that the toads can detect an object that adds only 3.1×10^{-12} $R^* s^{-1}$ to the ongoing rate of 4.9 ×

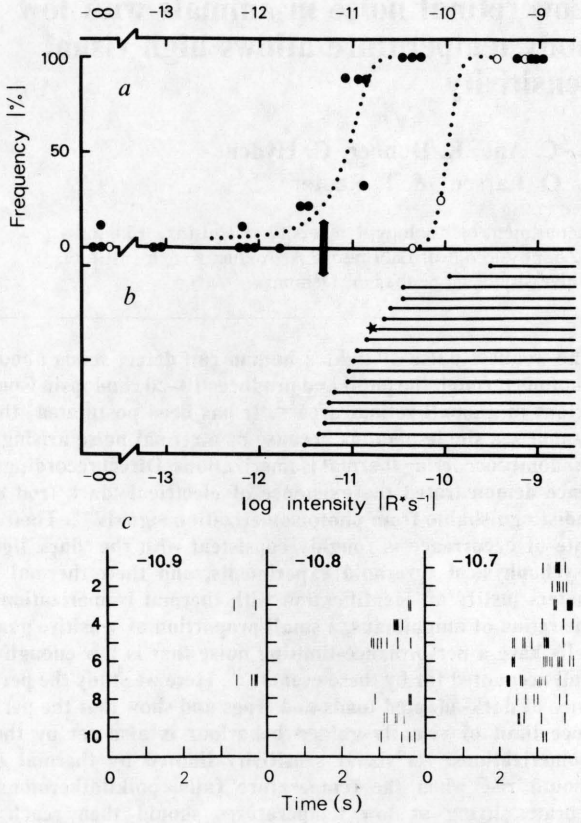

Fig. 2 Behavioural (*a*) and electrophysiological (*b, c*) determinations of absolute sensitivity at ~15 °C. The abscissa in (*a*) and (*b*) gives the intensity of illumination in the retina as numbers of photoisomerizations per rhodopsin molecule per second ($R^* s^{-1}$; logarithmic scale). Retinal stimulus in all cases (except for human psychophysics) was 0.39 mm^2 rectangle of light covering 4,500 rods. The thermal isomerization rate ($4.9 \times 10^{-12} R^* s^{-1}$) is marked by black arrows on the abscissa and has been obtained from the rate of 'dark' rod events found in *Bufo marinus*[5], and corrected for temperature. *a*, Snapping behaviour of *Bufo bufo*: each filled circle represents an experiment with nine toads; a response frequency of 100 on the ordinate means that all nine (9/9) snapped within 120 s after being placed in the apparatus (Fig. 1). Human psychophysics (open circles): frequency-of-seeing function determined in the same apparatus. The function is placed so that the abscissa directly gives isomerization rates in the human retina. This entails a slight shift in relation to the toad function, so that the 12-fold difference at the 50% response level corresponds to an 8-fold difference in the corresponding light intensities illuminating the dummy. The subject (A.-C.A.) aged 24 years, was given 60 min to dark-adapt; the cornea–dummy distance was 160 mm, her head was fixed but the eyes moved freely. The light source (P in Fig. 1) was shielded from view and the pupil area was 37 mm^2. 100 tests were performed in semi-random order: 20 at each of four intensities and 20 in darkness. The criterion for seeing corresponded to a 'glimpse of something' during a 25 s concentrated binocular search while two dummies passed, with a 30–60 s pause between searches. For the human subject, the test size corresponded to 1.1×7.2 degrees of visual angle (0.61 mm^2 on the retina) and was thus sufficient for full scotopic spatial summation. Assuming that 22% of 525 nm photons entering at the cornea produce isomerizations[2], and taking full dark-adapted spatio-temporal summation as 0.185 s deg^2 (ref. 17), the 50% threshold corresponds to 14 isomerizations in one retina, in good agreement with thresholds obtained with small flashed spot stimuli[1,2]. For calculation of human intensities in terms of $R^* s^{-1}$, see legend to Fig. 3. The two dotted curves are cumulative Poisson curves with thresholds and noise values determined for maximum-likelihood fit to the points.[7,8,10]. *b* and *c*: extracellularly recorded ganglion cell spike responses from isolated eyecups[8]. *b*, Horizontal lines give the ranges of stimulus intensities for which each of 18 cells responded to the rectangle; the small terminating dot in each case marks the intensity to which the cell responded on 50% of the trials. This 50% threshold turned out to be independent of whether the rectangle was moved across the receptive field at 'dummy-speed', or whether it was presented as an on-step of light. *c*, Response variability around threshold of the cell marked with a star in *b*. Here step stimulation was used to obtain precise time-courses. The three panels show responses to ten presentations of one fixed intensity each (log intensity in $R^* s^{-1}$ indicated in the upper left-hand corner). Each row of spikes shows one discharge on a time abscissa; stimulus onset at the left edge of the panel. The cell is seen to respond to all presentations of the highest intensity, but only twice to the lowest intensity, which is thus sub-threshold. This medium-sensitive cell had a very low maintained activity, giving only 5 'spontaneous' spikes during 1 h of recording.

$10^{-12} R^* s^{-1}$ from thermal isomerizations. But the limit to detection is set by random (Poisson) fluctuations in the number of isomerizations summed by a detector within consecutive counting intervals (summation times), not by rates as such. To evaluate whether thermal events really limit detection, it is necessary to calculate signal-to-noise ratios based on the relevant numbers[12]. For ganglion cells, summation areas and times are easily defined and the calculation is straightforward[7–10]. The most sensitive cell in Fig. 2*b* had a summation time of 1.9 s and collected signals from 440 rods (each containing about 3.25×10^9 molecules of rhodopsin). Thus at threshold it collected a mean number of 8.4 photoisomerizations, together with 13.3 thermal isomerizations, giving the upper limit $8.4/(8.4+13.3)^{1/2} = 1.8$ for the signal-to-noise ratio of the response. This indicates a low reliability of detection[13].

The brain of the toad may improve on the poor signal-to-noise ratio by summing signals from several sensitive ganglion cells. Uncertainties regarding the extent of central summation, sampling of the visual field, the role of binocular vision and so forth, preclude a strictly quantitative treatment, but it is instructive to consider the border conditions. The dummy image covered ~4,500 rods; within the summation time 1.9 s used above (the average among the 18 ganglion cells was in fact somewhat shorter at 1.5 s), the mean number of photoisomerizations collected over the whole image would be 86. During the same summation time the mean number of thermal isomerizations within the same area is 136. But the brain has no means of restricting its attention to that particular area and must sample the entire retina, which is 130 times larger.

Truly noise-limited performance must deteriorate if the noise increases, and this yields an interesting and directly testable prediction. The rate of thermal isomerizations grows about 4-fold for every 10 °C temperature rise[5]. If this noise constitutes a limiting factor, then the lowest possible threshold (in terms

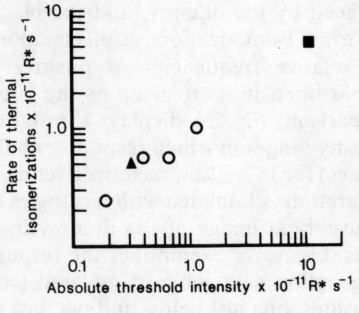

Fig. 3 Correlation between rates of thermal rhodopsin isomerizations (ordinate) and absolute threshold intensities (abscissa), expressed as rates of isomerizations per rhodopsin molecule in the retina of the toad, *Bufo bufo* (▲), the frog, *Rana temporaria* (○), and man (■). The toad (15 °C) and human (37 °C) results are taken from Fig. 2*a*; the frog results were obtained at temperatures (from left to right) 10 °C, 16 °C, 16 °C and 20 °C. For methods, see ref. 9. For proper appreciation of the differences, note that the sensitivity of frog rods (response amplitude/photoisomerization) is in fact lower at 10 °C than at 16 or 20 °C (ref. 18). For the amphibians, threshold is the lowest intensity for which the light-dependence of the behaviour was statistically significant ($P < 0.01$). In the phototaxis experiments with *Rana*, the spacing of trial intensities was ~0.3 log units. The human threshold (50% responses) is in good agreement with the generally accepted value of 10–20 quanta used[2], if full dark-adapted spatio-temporal summation is assumed[17]. Amphibian rates of thermal isomerizations are taken from ref. 5 and corrected for temperature; human rate of thermal isomerizations is from ref. 6. Human photoisomerizations per rhodopsin molecule have been calculated assuming there are 1.2×10^8 molecules per rod[6] and a peak rod density of 160,000 per mm^2 retina[19].

of $R^* s^{-1}$) of animals active at high body temperatures should be higher than that of our toads at 15 °C (provided that temporal summation is not significantly larger in warmer animals).

To test this idea, we have determined absolute thresholds in man, and frogs at different body temperatures. Again, we expressed the thresholds in terms of photoisomerizations per rhodopsin molecule per second, thus eliminating all differences in optics anatomy of the rods and compared them with the estimated rates for thermal isomerizations. The human thresholds were determined with the same prey-dummies in the same apparatus as was used for the toads; the frequency-of-seeing function of one subject is plotted as open circles in Fig. 2*a*. For frogs, we determined the absolute threshold for the phototactic jumping behaviour at three different temperatures[9]. These results are summarized in Fig. 3 as a log-log plot of threshold intensities against rates of thermal isomerizations.

A monotone relation is found between threshold intensity and rate of thermal isomerizations. This is consistent with the hypothesis that thermal isomerization of rhodopsin sets the ultimate limits on threshold intensity for the visual detection task. This could be ecologically significant, for example for vision in the cold and dark environment inhabited by deep-sea fishes. It also emphasizes the functional importance of any anatomical structures that increase the numbers of photons absorbed without increasing the amount of absorbing pigment (the number of noise-producing rhodopsin molecules). Well known examples of such structures are reflecting tapeta and light-funneling inner segments of photoreceptors.

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