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CAN A SHORE CRAB SEE A STAR?

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SUMMARY

1. The absolute-intensity threshold of the optokinetic response in the crab *Leptograpsus variegatus* was measured using a moving continuous monochromatic point source. The results are compared to the photoreceptor responses of the same animal (Doujak, 1984), to determine the photoreceptor signal for the same behavioural threshold stimuli.

2. Optokinetic eye movements demonstrate that at the peak of spectral sensitivity (499 nm), the minimum intensity of light the animal can detect is $4{\cdot}0 \pm 1{\cdot}5 \times 10^5$ photons $cm^{-2} s^{-1}$ (mean ± S.D., $N = 18$) incident on the eye, which is equivalent to a photon flux of about 6 photons $facet^{-1} s^{-1}$.

3. Comparison of behavioural and electrophysiological studies shows that at the above behavioural thresholds, the retinula cells respond with a train of discrete membrane depolarizations (bumps). The mean bump rate recorded in retinula cells at the absolute-intensity threshold of the optokinetic response to a moving point source is 22 ± 5 bumps min^{-1} (mean ± S.D., $N = 6$).

4. Optokinetic experiments reveal an absolute sensitivity of the crab's apposition eye to a point source that is only about 900 times less sensitive than the human eye: theoretical estimates based on quantum capture efficiency and lens size predict a much larger difference. The experimental findings provide the first definitive proof that an animal possessing a compound eye can see a star, albeit only stars of 0·5 magnitude and brighter.

INTRODUCTION

One measure of a visual system's performance is its ability to detect point sources of low intensity. For the human visual system Hecht, Schlaer & Pirenne (1942) determined that a minimum of 5–10 photon absorptions within 0·1 s are necessary to produce a sensation of light. The absolute-intensity threshold would be roughly equivalent to the earlier observations made by astronomers that an observer can see a star of 8th magnitude under ideal conditions (Curtis, 1901).

The minimum intensity of a point source that a given visual system can perceive is largely set by the size of its lens, which limits the photon flux available to the photoreceptors. Since facets in compound eyes are smaller than the pupil in lens eyes,

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it has generally been accepted that compound eyes have a poor absolute sensitivity to point sources (Barlow, 1952; Rodieck, 1973, p. 272; Kirschfeld, 1974). Theoretical comparison of the absolute sensitivity of lens and compound eyes based on lens size differences led Rodieck (1973) and Kirschfeld (1974) to conclude that an animal possessing an apposition eye should not be able to perceive even the brightest 'star' in the night sky. However, Horridge (1966*b*) suggested that some of the brighter stars might be seen though his experimental findings do not support this.

In spite of the commonly held assertion that animals with compound eyes are virtually blind to the richness of the night sky, no behavioural light threshold for point sources, apart from an early attempt by Horridge (1966*a*), has yet been established. Although numerous behavioural experiments have confirmed the high absolute sensitivity of insect optomotor systems, demonstrating that intensities which produce single photon responses are adequate sensory stimuli (Fermi & Reichardt, 1963; Reichardt, 1969; Scholes & Reichardt, 1969; Lillywhite & Dvorak, 1980; Dubs, Laughlin & Srinivasan, 1981), all the stimuli employed have been extended sources. The demonstration of spatial summation at low light intensities as a mechanism to increase photon capture (Dubs *et al.* 1981) makes it difficult to estimate the absolute-intensity threshold for a point source from threshold experiments using extended sources.

A recent electrophysiological investigation of a crustacean photoreceptor revealed discrete membrane depolarizations (bumps) in response to absorption of single isolated photons at low light intensities (Doujak, 1984). The aim of the present report is to establish the absolute minimum intensity of a point source that a crustacean visual system can detect and to compare this with the previous electrophysiological study to determine the photoreceptor signal at behavioural threshold. Factors that may account for the discrepancy between the high absolute sensitivity measured behaviourally and the low absolute sensitivity predicted from the small entrance pupil of a crab's eye are also discussed.

MATERIALS AND METHODS

Optokinetic experiments were carried out on the Australian shore crab, *Leptograpsus variegatus* Fabricius. The animals were maintained at 20 °C in a closed sea water (SW) aquarium. The photoreceptive membrane in *Leptograpsus* is known to be actively involved in resynthesis, producing a larger rhabdom at night (Stowe, 1980). Since the effect of a natural dusk or dawn (twilight) on the magnitude of microvillar membrane resynthesis is not yet known, the aquarium was kept by a window in an otherwise dark room to ensure optimal matching of the animal's natural light environment.

Optokinetic eye movements evoked by a moving point source in an otherwise dark room were recorded in intact animals. Measurements were made from animals without regard to sex and/or size. All experiments were prepared during dusk and carried out 2–7 h after dusk to ensure measurement of absolute light sensitivity of dark-adapted night eyes.

The method used to record excursions of the eye due to tracking of the light source is illustrated in Fig. 1. Crabs were positioned at the centre of a perimeter device by a perspex holder glued to the back of the carapace, and allowed to walk on a styrofoam ball floating in a SW-filled beaker. An accurate measure of the angular excursion of the eye was provided by a lightweight flag (thin black photographic paper attached to the end of a thin silver wire) glued to the top of the eyestalk so that it projected over the back of the animal. The flag imposed a negligible load (0·015 g) on the eye and did not affect the eye movements or occlude vision. The flag was interposed between an infra-red LED (880 nm) and a silicon photodetector. Optokinetic eye movements cause a displacement of the flag, exposing a variable area of the photodetector to the infra-red beam. The LED and photodetector were initially aligned so that the detector was completely occluded when the eye was in its natural alert seeing position. Eye movements were monitored by the output of the photodetector; the larger the displacement of the eye from the longitudinal axis of the animal the larger the output recorded from the photodetector. The above method could reliably detect angular excursions of the eye as low as 1·0° and overall excursions as large as 90° to either side of the animal.

The light stimulus was a monochromatic (499 nm) point source obtained by passing light from a tungsten-iodide bulb (GE 46) through a narrow-band spectral interference filter (Schott) upon one end of a quartz-fibre optic light guide. The

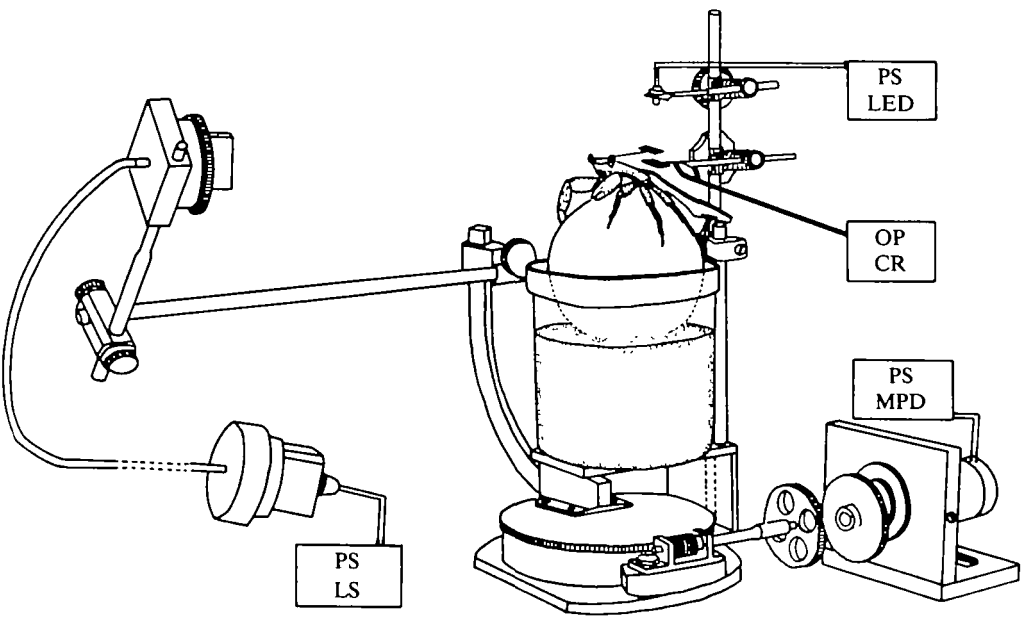

Fig. 1. Experimental arrangement used to record the optokinetic eye movements evoked by a moving point source. Power supply (PS) for the stimulus light source (LS), motorized perimeter device (MPD) and infra-red light emitting diode (LED). The output from the photodetector (OP) is recorded on a chart recorder (CR).

interference filter was checked for undesirable secondary transmission, which would overestimate radiometric calibrations of the effective spectral stimulus intensity. The interference filter was checked between 200 and 800 nm (spectral response of photomultiplier) on a Shimadzu Graphicord spectrophotometer and had a peak transmittance at 499 nm with a half band-width of 8 nm and contained no side band transmittance. The light source and filter were encased within a light-tight aluminium holder allowing light to leave only through the light guide. The intensity of the light source was controlled by adjusting the output of a d.c. power supply. The stimulating end of the light guide was fixed on a Cardan arm 27 cm in front of the animal, supplying virtually parallel rays to the crab's eye. The stimulus was effectively a point source, subtending 0·2° at the cornea. For threshold experiments using polarized light, a holder was fitted to the stimulating end of the light guide, allowing a polaroid filter (type HN22) to be interposed between the light guide and the animal. All the experiments were carried out in a dark room, where the only light available to the animal was that provided by the stimulus.

The perimeter device was motorized to provide smooth movement of the light source horizontally about the animal. A range of velocities could be selected, but in all the experiments to be described, a velocity producing an angular displacement of approx. $180° h^{-1}$ about the animal was chosen.

The intensity of the stimulus incident on the crab's eye was calibrated *in situ* using an International Light radiant flux detector (PM 270D) with an IL 760 photomultiplier power supply and an International Light research radiometer (700). The PM 270D photomultiplier has a high radiometric efficiency, resolving 1×10^{-13} W cm^{-2} at 500 nm, and could measure the dimmest light used in the experiments. The light intensities were measured in W cm^{-2} at 499 nm and converted to equivalent photons cm^{-2} s^{-1} at 499 nm, with appropriate corrections for the photomultiplier's spectral sensitivity applied. The photomultiplier and radiometer were standardized by International Light Inc. whose radiometric calibrations are traceable to the National Bureau of Standards in Washington, D.C. The combined measurement accuracy of the instruments is ±2·0 %. The stability of the light source was tested over the duration of the experiments and showed no significant signs of change in its output.

RESULTS

Optokinetic eye movements in crabs can be evoked by movement of a striped drum or light-spot about the midline axis of a stationary animal, provided the stimulus is the only object on which the eye can stabilize (Horridge & Sandeman, 1964; Horridge, 1966*a*). The animal will move its stalked eyes at a velocity and in a direction which are related to the movement of the visual stimulus. Previous studies in *Leptograpsus* have demonstrated that the lateral-equatorial ommatidia are the most sensitive to movement of a visual stimulus (Sandeman, 1978). Therefore in the present study, to ensure that the absolute-intensity threshold of the optokinetic system was recorded,

the point source stimulus was aligned with respect to the animal so that the equatorial region of the eye was illuminated. The eye movements were monitored continuously throughout the experiments.

In all the experiments the light source moved through an angle of about 90° to either side of the animal and at a constant velocity of $3{\cdot}0°\,min^{-1}$. Movement of the light source generally began at the front of the animal and after having moved through 90°, was followed by movement in the opposite direction. Prior to each run the intensity was set and remained constant throughout the full angular excursion of the light. At or near absolute threshold intensities, a minimum of eight responses for each intensity were measured.

Two main types of eye movements occur in *Leptograpsus*: a phasic and a tonic eye movement. The tonic eye movement (slow nystagmus) predominates when the eye is tracking a steadily-moving visual field (light-spot in a dark room) and the phasic eye movement (fast nystagmus) is characteristic of the return of the eye to its normal alert seeing position. Angular displacements of the eye more than 10° during the slow nystagmus phase are typically interrupted by a phasic eye movement. In response to supra-threshold stimuli, movement of the light source about the animal will result in a sequence of saccades (Fig. 2).

The optokinetic experiments demonstrated that the minimum intensity required to evoke a response is at least three times higher at the front of the eye than at the side. At the absolute minimum intensity, only movement of the light source at the side of the animal would evoke a response. This agrees with the observation by Sandeman (1978) that the lateral-equatorial ommatidia are the most sensitive to horizontal movement, although Sandeman simply showed that larger eye movements are evoked when the stimulus was at the side of the animal. The differences in sensitivity to horizontal movement may in part account for the low sensitivity measured by Horridge (1966*a*),

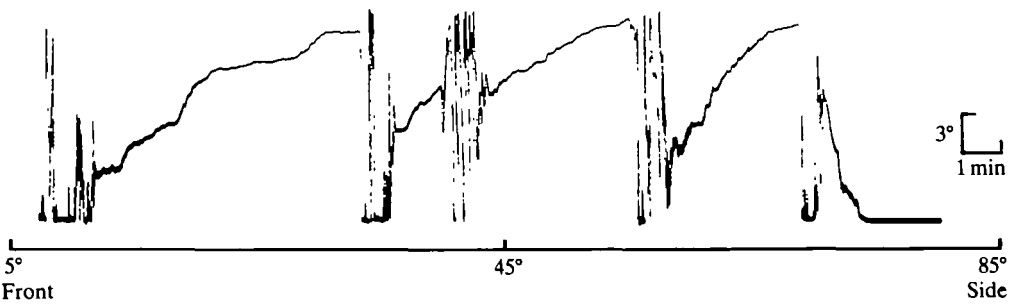

Fig. 2. Optokinetic response at supra-threshold light intensity (photon flux about 150 photons facet^{-1} s^{-1}). Large displacements of the eye from the midline axis of the animal, resulting from tonic eye movements, are followed by phasic eye movements that reposition the eye to its normal alert seeing position. At supra-threshold light intensities movement of the light source about both the front and side of the animal will evoke a response. Thus movement of the light source from the front of the animal through 90° to the side of the animal will result in a sequence of saccades as recorded above. The lower trace shows the stimulus position with respect to the eye.

since only the absolute threshold of the frontal eye regions in his animals were measured. The differences between frontal and lateral regions of the eye could arise from differences in facet diameters and/or interommatidial angles, differing intensity thresholds of two independent optokinetic systems, or relative weighting of information from different points in the visual field upon a common optokinetic interneurone. The constancy of facet diameters and interommatidial angles along the horizontal meridians of the eye (Sandeman, 1978) and the observation that wide field stimuli produce more reliable and stronger optokinetic responses (Sandeman & Erber, 1976), implying considerable spatial summation across the eye, suggest that the latter mechanism is most likely. A lower threshold for the lateral-equatorial ommatidia could result from preferential amplification of their signals, as a consequence of the location and density of their synapses. Spatial weighting of local motion information by synaptic density has been previously suggested as a mechanism to account for the spatial sensitivity profiles of motion sensitive interneurones in the fly (Hausen, Wolburg-Buchholz & Ribi, 1980).

Tonic optokinetic eye movements evoked by movement of a light stimulus in an otherwise dark room provided a method for identifying the minimum intensity of light the animal could see. The criterion used to establish the absolute-intensity threshold was the minimum intensity the animal could track the stimulus for at least a total angular displacement of 8° across the eye. At or near threshold the intensity was progressively increased in approximately 0·5 log unit increment steps until the minimum intensity required to evoke a slow optokinetic following response could be ascertained. The intensity was then calculated from the calibration.

The minimum intensity of light that would evoke an optokinetic response was found to be approx. $4{\cdot}0 \pm 1{\cdot}5 \times 10^5$ photons $cm^{-2} s^{-1}$ (mean ± S.D., $N = 18$) at the eye and is equivalent to a photon flux incident on the cornea of approx. 6 photons $facet^{-1} s^{-1}$ (hexagonal facet diameter = 45 μm). The threshold intensities were the same for crabs of all sizes and both sexes. At or near threshold, the overall optokinetic response was weaker, with the eye not being able to track the movement of the light reliably (Fig. 3). The difference in velocity between the tonic eye movement and the stimulus increased at threshold intensities, demonstrating that the strength and magnitude of the response are related to the effectiveness of the stimulus. At low light intensities, quantum uncertainty in the stimulus due to the Poisson nature of light will be an important factor governing the deterioration of the optokinetic response.

A recent electrophysiological study of retinula cells in *Leptograpsus* has revealed the presence of discrete membrane depolarizations (bumps) in response to appropriate low light intensities (Doujak, 1984). Statistical analysis of bump recordings is consistent with the hypothesis that bumps represent the photoreceptor's response to single photon absorptions.

Comparison of behavioural and electrophysiological studies demonstrates that retinula cells, stimulated by a point source on axis and with an intensity equal to behavioural threshold, respond with a train of bumps at a rate of $22 \pm 5\ min^{-1}$ (mean ± S.D., $N = 6$) (Doujak, 1984). However, the absolute number of bumps per unit time which stimulates a higher order optokinetic neurone will depend on the

extent of spatial summation between retinula cells, both within and between ommatidia. Although the use of a point source eliminates considerable spatial summation between neighbouring facets, in the dark-adapted night eye where the interommatidial angle is 1·5° (Sandeman, 1978) and the angular acceptance function is 3·5° wide at the 50 % level (personal observations), the nearest and second nearest facets will still be approx. 60 % and 20 % as sensitive as the one receiving light on axis. To determine whether retinula cells within the same ommatidium are providing input to the optokinetic system, optokinetic experiments using polarized light were carried out.

Retinula cells in *Leptograpsus* are polarization sensitive and respond maximally when the plane of polarized light is orientated either horizontally or vertically with respect to the horizon when the eye is in its normal alert position (Doujak, 1984). The polarization sensitivity peaks correspond to the orientation of the retinula cells' microvilli. In crustaceans, the tiered rhabdom is made up of microvilli contributed by three retinula cells which alternate with orthogonally orientated microvilli contributed by four other retinula cells. If only one set of retinula cells contributes to the optokinetic system, one E-vector orientation would require an intensity five times brighter than the other to evoke an equivalent response (dichroism = 5; Doujak, 1984).

Optokinetic experiments using polarized light ($N = 7$) showed that the intensity threshold is not influenced by rotation of the plane of polarization, indicating that the optokinetic response is driven by input from both sets of retinula cells within an ommatidium. Assuming spatial summation of retinula cell responses, both within and between ommatidia viewing the point source, the total effective signal can be estimated to consist of, on average, 18 bumps s^{-1} (i.e. signal/retinula cell × 7 retinula cells/facet × 7 from summed contribution of off-axis ommatidia).

DISCUSSION

Since the light-gathering power of a lens is proportional to the area of its aperture and because facets in compound eyes are small, it has become a widely accepted view that compound eyes are a poor device for the detection of point sources (Barlow, 1952;

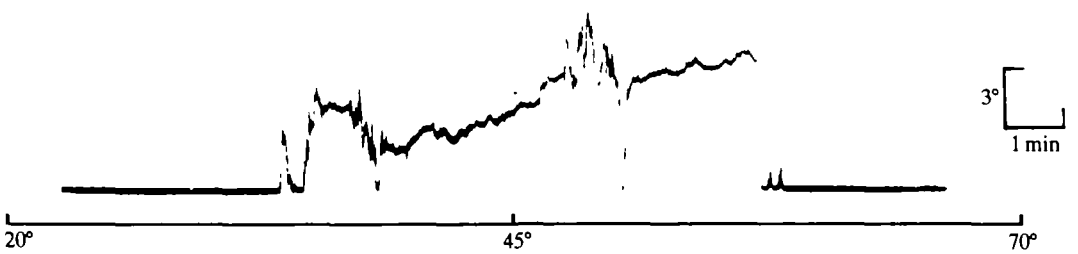

Fig. 3 Optokinetic response at behavioural threshold light intensity (photon flux about 6 photons facet^{-1} s^{-1}). At threshold light intensities, only movement of the light source at the side of the animal will evoke a response. The lower trace shows the stimulus position with respect to the eye.

Rodieck, 1973; Kirschfeld, 1974). A comparison of the size of hexagonal facets in *Leptograpsus* (45 μm) and the size of the dark-adapted pupil in humans (8 mm, p. 104 Le Grand, 1957) would suggest that for point sources, the human eye is about 33 000 times more sensitive than an apposition eye. However, not all incident photons are absorbed by the photoreceptors; hence a comparison of the absolute sensitivities of lens and compound eyes must be corrected for the quantum capture efficiency (QCE) of the respective types of eyes. Estimates of human QCE yield values between 5 and 10% (Hecht *et al.* 1942; Barlow, 1977). In *Leptograpsus*, electrophysiological measurement of QCE yields values of approximately 45% (Doujak, 1984). Thus, the absolute sensitivity of an apposition eye to point sources would be expected to be about 7200 times less than that of the human eye.

The present study demonstrates the minimum intensity of a point source at 499 nm that will elicit an optokinetic response is approx. $4 \pm 1{\cdot}5 \times 10^5$ photons $cm^{-2} s^{-1}$ incident on the crab's eye. Optokinetic experiments reveal an absolute sensitivity of the crab eye that is only about 900 times less than that of the human eye, exceeding theoretical expectations based on the above considerations (lens size and QCE) by a factor of eight. This figure was derived by comparing the intensity of a continuous light source needed to elicit a sensation of light in the human and crab respectively. The smallest energy flux detectable by the human eye for a steady, effectively point, source of light is about 442 photons $cm^{-2} s^{-1}$ at 507 nm (Marriott, Morris & Pirenne, 1959).

The most likely mechanisms to account for the discrepancy between the behavioural measurements and the theoretical estimate of the absolute sensitivity of an apposition eye to a point source are spatial and temporal summation. The theoretical estimate assumes that a point source is viewed by only a single facet. However, motion-sensitive interneurones so far characterized in *Leptograpsus* are all wide-field units (Erber & Sandeman, 1976) and stronger optokinetic responses are evoked when wide-field stimuli are employed (Sandeman & Erber, 1976). Therefore, there must be considerable spatial summation across the eye, so that input from off-axis ommatidia viewing a point source cannot be neglected. A theoretical calculation of the absolute sensitivity of the crab eye would yield a value of approximately 1000 times less than that of the human eye when spatial summation is also considered (i.e. the effective quantum capture area corrected to unity is seven times larger than a single facet). Alternatively, temporal summation could account for the discrepancy. If there is a threshold (i.e. requirement for the cumulative effects of more than one quantum capture per unit time) then at low light intensities when photon absorptions become separated, on average, by longer time intervals a greater sensitivity will be afforded to an animal with a longer visual interneurone summation time. Support for a longer summation time in the crab than in man comes from the demonstration that the crustacean eye can perceive movements as slow as $15\,° h^{-1}$ (Horridge & Sandeman, 1964; Sandeman & Erber, 1976), because a longer summation time also reduces the threshold velocity.

What does the shore crab's absolute intensity threshold for a point source tell us with respect to the magnitude of stars it can potentially see? The crab apposition eye

being only about 900 times less sensitive than the human eye removes any doubt that the crab can see stars of 0·5 magnitude or brighter, under ideal conditions. Albeit, not more than about 12 stars will be within the visual limits of the crab, the absolute-intensity threshold does attest to the remarkably high performance of an apposition eye and demonstrates that information can be derived from photoreceptor signals consisting of quantum bumps.

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