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RESEARCH ARTICLE

Cockroach optomotor responses below single photon level

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ABSTRACT

Reliable vision in dim light depends on the efficient capture of photons. Moreover, visually guided behaviour requires reliable signals from the photoreceptors to generate appropriate motor reactions. Here, we show that at behavioural low-light threshold, cockroach photoreceptors respond to moving gratings with single-photon absorption events known as 'quantum bumps' at or below the rate of 0.1 s^{-1}. By performing behavioural experiments and intracellular recordings from photoreceptors under identical stimulus conditions, we demonstrate that continuous modulation of the photoreceptor membrane potential is not necessary to elicit visually guided behaviour. The results indicate that in cockroach motion detection, massive temporal and spatial pooling takes place throughout the eye under dim conditions, involving currently unknown neural processing algorithms. The extremely high night-vision capability of the cockroach visual system provides a roadmap for bio-mimetic imaging design.

KEY WORDS: Behaviour, Intracellular, Optomotor, Photoreceptor

INTRODUCTION

Animals experiencing rotation of their environments will start rotating in the same direction in order to stabilize their vision. This optomotor response is a compensatory mechanism for unintended deviations from the animal's course of motion (Egelhaaf and Borst, 1993; Srinivasan et al., 1999). Our experimental animal, the American cockroach (*Periplaneta americana*, Linnaeus 1758) is a nocturnal insect with large apposition-type compound eyes. Although vision in the cockroach is traditionally thought of as a less important sensory modality than mechanosensation (Baba et al., 2010) and olfaction (Willis et al., 2008), it mediates circadian rhythms (Roberts, 1965), the shade response (Okada and Toh, 1998) and antennal guidance (Lent and Kwon, 2004; Ye et al., 2003). Because cockroaches are dark active, we used them as a model to search for such a behavioural dim-light threshold, where they would still respond to rotating wide-field stimulation with the optomotor reaction. This threshold was calibrated with intracellular photoreceptor recordings under identical stimulus conditions.

Although the optomotor reaction of the American cockroach has been known since the 1950s (Autrum and Stöcker, 1952), this research paradigm could be problematic with freely moving cockroaches, which tend to abandon visual navigation when tactile (Baba et al., 2010) or chemosensory (Willis et al., 2008) cues become available. We were able to overcome this problem with a panoramic virtual reality (VR) system (Takalo et al., 2012) that was fitted with a trackball. Carefully positioning the cockroach on the trackball could eliminate inputs from the antennae and mouthparts, and create conditions under which it was constantly motivated to walk under visual stimulation (Fig. 1A,B). The VR system enabled quantified behavioural observations of temporal and spatial summation in the cockroach visual system. Temporal summation means the slowing down of the photoreceptors and/or pooling of photoreceptor signals of one channel over time in higher visual areas. As a result, the ability to see faster moving objects gets worse. Spatial summation means the pooling of signals from several channels (such as from several ommatidia that form the 'pixels' in compound eye vision), or, in the case of single photoreceptors, collecting light from a wider visual angle. Spatial summation results in decreasing image resolution and loss of finer details (Warrant, 2006).

The stimulus we used in the VR system was a black-and-white grating, rotating horizontally with nine different temporal frequencies (see supplementary material Movie 1). The relationship between the angular distance moved by the cockroach in the direction of the movement of the stimulus and the total angular distance (Strauss et al., 1997) it covered during the experiment was used to quantify the response strength. Stationary gratings were applied as controls. Stimulus light intensities that elicited optomotor reactions were between 0.005 and 500 lx, corresponding to moonless night sky and typical office lighting, respectively. For comparison, the illuminance of the sky measured in clear sunlight can be up to 100,000 lx (Bond and Henderson, 1963; Cronin et al., 2014).

RESULTS

Experiments with different temporal frequencies (Fig. 2A–D), as seen by the photoreceptors, showed increasing temporal summation with lowering light intensity, when judged on the basis of the optomotor response. The average control values were close to the expected 0, indicating random movement in both directions. A value of 0 was always obtained at the highest temporal frequency (18 Hz) and for most frequencies at the lowest light intensity that caused any reactions (0.005 lx). At high intensities, the band of temporal frequencies eliciting the optomotor response was wide and narrowed towards 0.005 lx. The response strength at 5 and 500 lx increased with temporal frequency from 0.1 to 4 and 6 Hz, respectively, and declined with higher frequencies (Fig. 2A,B). The response to the moving grating patterns was significantly different to that to the stationary gratings (controls) up to 15 Hz ($P<0.05$). At 0.05 lx, cockroaches followed the grating patterns of all temporal frequencies up to 10 Hz (Fig. 2C), but at 0.005 lx only at frequencies between 0.4 and 4 Hz (Fig. 2D). With one decade lower light intensity, at 0.0005 lx, the response disappears altogether (Fig. 3A). We can interpret these findings so that the time constant ($\tau=1/2\pi f$) of the integrator in motion detection increases from approximately 10 ms (15 Hz) to approximately 40 ms (4 Hz).

Behavioural experiments also showed evidence of increasing spatial summation when the visual environment became darker. Fig. 2E–H shows pooled data from experiments with different angular periods (angular wavelengths) of the stimulus pattern. The data show a similar attenuation of the response with falling light

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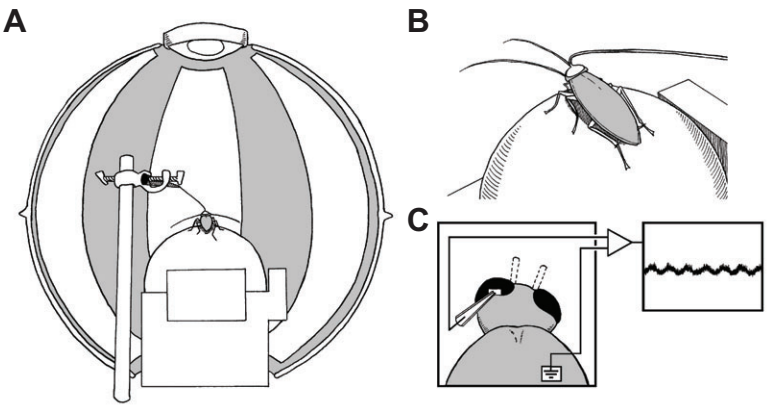

Fig. 1. The virtual reality setup. (A) A fisheye lens on top projects the images of black and white bars from the projector above it (not shown) onto the inner surface of the spherical screen. The trackball system is placed inside the sphere so that the cockroach is at the equator of the sphere. (B) The placement of the animal on the trackball. (C) Electrode placement in the preparation during intracellular recordings. Dashed line shows where the antennae of an intact cockroach would be. For more detailed information, see Takalo et al. (Takalo et al., 2012).

levels as the temporal frequency data (Fig. 2A–D). The lowest light intensity where significant responses were found was 0.005 lx (Fig. 2H), although here the pooled responses between 0.4 and 4 Hz of the stimulus with a 60 deg angular period did not significantly differ from the control. At one decade lower light intensity of 0.0005 lx we found no responses to any of the angular periods (Fig. 3B). The responses at 500 lx and 5 lx were very similar, but at 0.05 lx the narrow patterns of 20 deg and 40 deg began to be attenuated and at 0.005 lx the cockroaches reacted only to 90 deg and 180 deg patterns. Therefore, we conclude that the optomotor response of the American cockroach persisted down to the illuminance of 0.005 lx, where, with 60 deg stimulus, the animal still

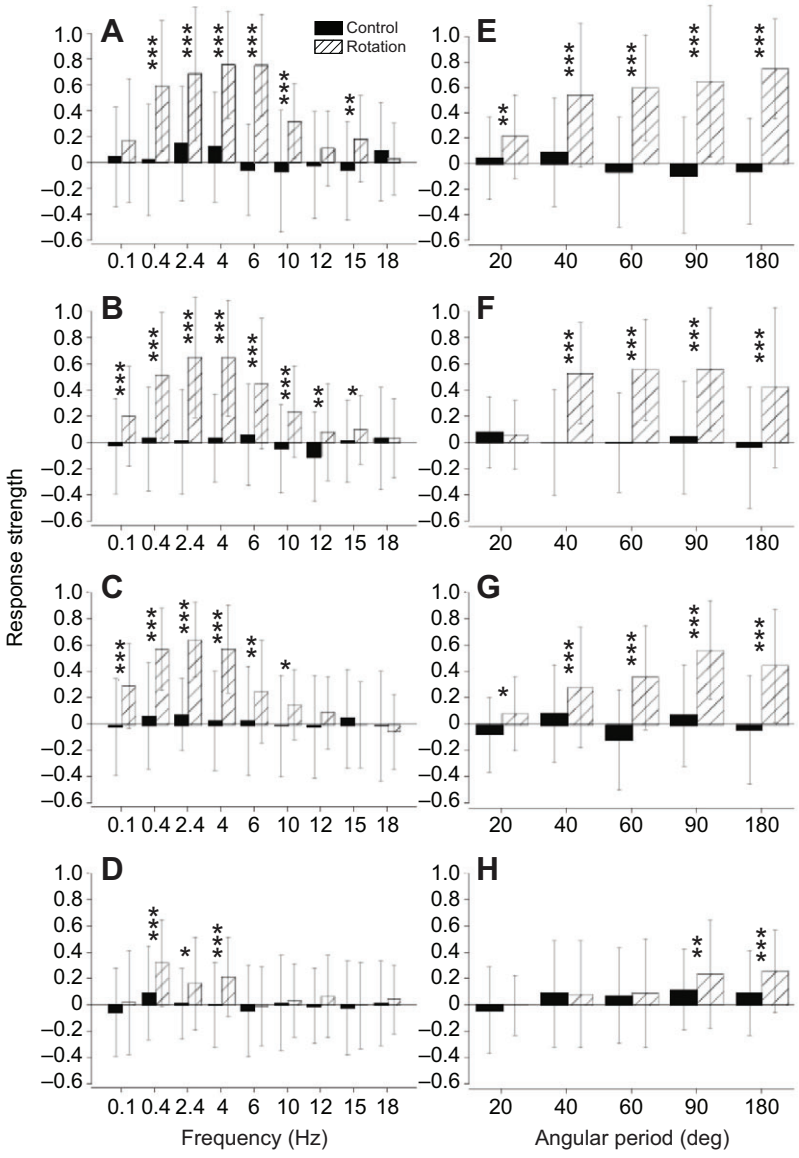

Fig. 2. Behavioural response strengths. The mean strengths of responses ± s.d. of cockroaches to optomotor stimuli at different temporal frequencies (A–D) and angular periods (E–H) of the rotation stimulus under (A,E) 500 lx, (B,F) 5 lx, (C,G) 0.05 lx, (D,H) 0.005 lx. Solid bars denote the response strength during stationary controls, and hatched bars represent that during rotating stimuli. The expected control level is 0; +1 indicates the strongest positive and −1 the strongest anti-directional response. *, **, *** indicate significance between the control and rotation distributions at confidence levels of 0.05, 0.01 and 0.001, respectively (paired sample Wilcoxon signed rank test). (A–D) The responses to rotation of the stimulus with a 60 deg angular period at different temporal frequencies were attenuated and their frequency band narrowed from the brightest (500 lx) to the dimmest (0.005 lx) stimuli. (E–H) The responses to different angular periods of the stimuli were compiled from another data set by pooling all responses to temporal frequencies between 0.4 and 10 Hz (E–G) or 0.4 and 4 Hz (H), the frequencies most likely to elicit the optomotor response according to results in A–D. (E) All angular periods were able to elicit the optomotor response at 500 lx illumination. Responses to angular periods of 40–180 deg remained significant down to 0.05 lx (F,G). The lowest light intensity at which significant responses were seen was 0.005 lx, although 60 deg stimuli did not elicit a significant response (H). Sample sizes were N=20 animals, n=40 measurements (A); N=24, n=78 (B); N=23, n=66 (C); N=23, n=66 (D); and N=8–12, n=48–174 (E–H). See also Fig. 3.

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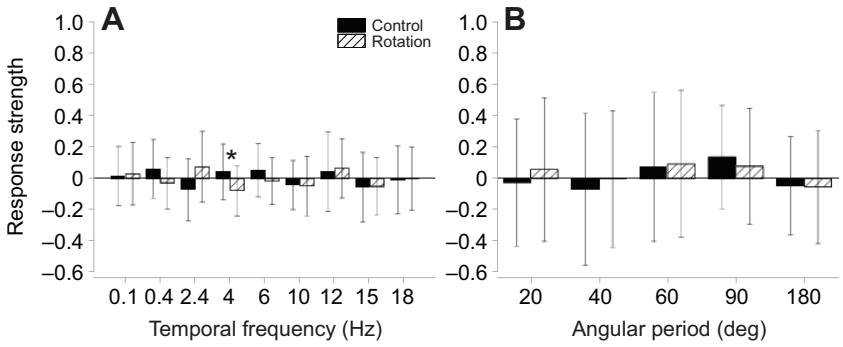

Fig. 3. The average strengths of behavioural responses ± s.d. of cockroaches to optomotor stimuli at 0.0005 lx. See Fig. 2 for key to the symbols. (A) The responses to rotation of the stimulus with a 60 deg angular period at different temporal frequencies. Both control and rotation values were scattered around 0, and the only significant difference at 4 Hz was a confounding factor of the small sample size. (B) Combined responses from temporal frequencies between 0.4 and 4 Hz at different angular periods of the stimulus. No significant differences between control and rotation values were found. Apparently, the absolute dim-light vision threshold of the American cockroach lies somewhere between 0.005 and 0.0005 lx. Sample sizes were *N*=11 animals, *n*=22 measurements (A); *N*=9–12, *n*=27–132 (B).

responded reliably to the same frequencies that caused the strongest responses at 5 lx intensity.

Intracellular recordings were made using the same VR setup (Fig. 1C), and they showed that the photoreceptor responses consisted of discrete single photon responses or 'quantum bumps' at the behavioural threshold. Similar results have been obtained previously in flies (Dubs et al., 1981; Scholes and Reichardt, 1969), but we are confident that our use of identical stimuli in both behavioural and electrophysiological experiments, along with the use of the spherical VR setup, gives more reliable results. All our recordings were made from green-sensitive photoreceptors, because the projector used in the VR setup did not emit ultraviolet (Takalo et al., 2012). First, 500 lx high-intensity stimuli were used to characterize photoreceptor light responses under VR settings. (Fig. 4A). Presenting a dark-adapted photoreceptor with a stationary control grating caused an initial transient response of 10–40 mV. Response amplitude varied with the sensitivity of the cell and whether the ommatidial optical axis pointed towards the black or the white bar of the stimulus grating. The photoreceptor response typically adapted close to the resting potential during the 30 s control, leaving vigorous photon shot noise (Warrant, 2006) on top of a 2–10 mV membrane depolarization. When the grating started rotating, graded responses to light modulation were found with amplitudes up to 8 mV, depending on the (varying) sensitivities of the cells (Heimonen et al., 2006).

Similarly, the control stimulation at 5 lx caused a transient 5–15 mV step response (Fig. 4B), which adapted close to the resting potential during the control, while small (<2 mV) depolarization remained with superimposed photon shot noise. With movement stimulation applied, some cells reacted to temporal frequencies of 0.1–6 Hz with a small graded response of 1–2 mV. The intracellular responses of the photoreceptors at 500 and 5 lx were thus quite

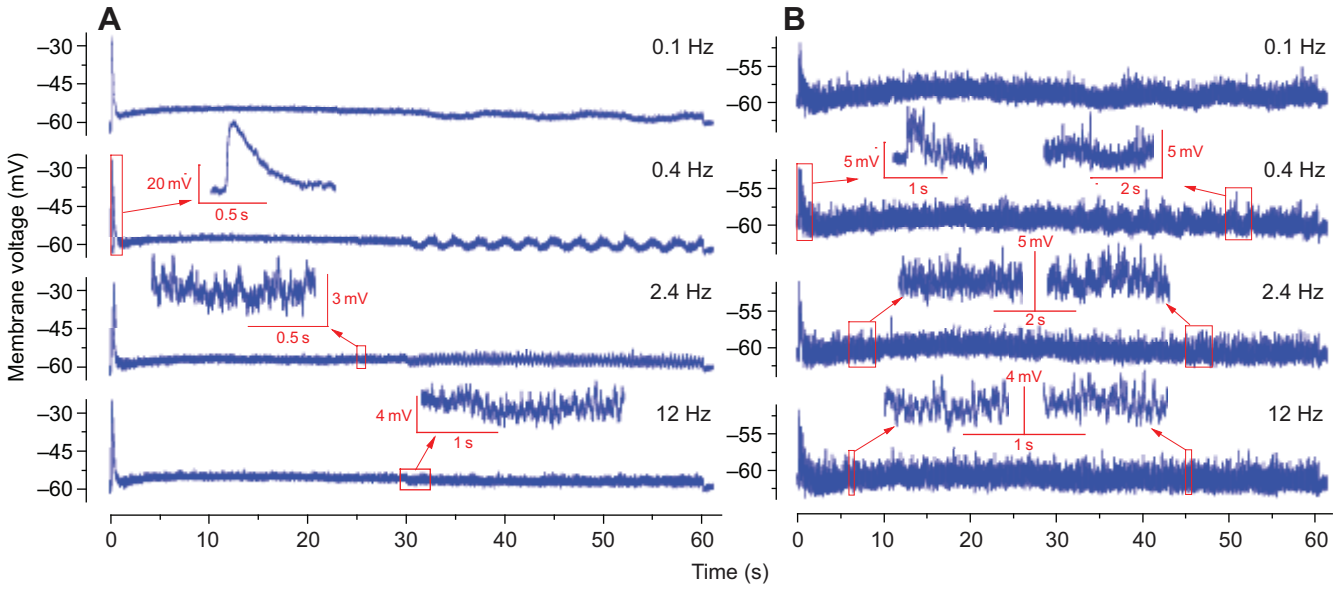

Fig. 4. Examples of light-on responses and graded responses to light modulation during rotation (30–60 s) in one photoreceptor cell. Stimulus frequency is indicated above each 500 lx (A) and 5 lx (B) recording. (A) Light-on responses adapt to plateau near resting potential at approximately 800 ms (inset image in the 0.4 Hz recording). Stimulus rotation caused a membrane voltage modulation. In 2.4 and 12 Hz recordings, a slight repolarization marks the onset of rotation (inset image at 12 Hz), because this cell faced white during the control. (B) The 5 lx recordings show small, <10 mV light-on responses (left inset image at 0.4 Hz). The modulation response is overlaid with photon shot noise (inset images at 0.4 and 2.4 Hz). In the 12 Hz recording, the modulation begins to be lost under the noise, but behavioural results show that an optomotor response at this frequency can still be possible.

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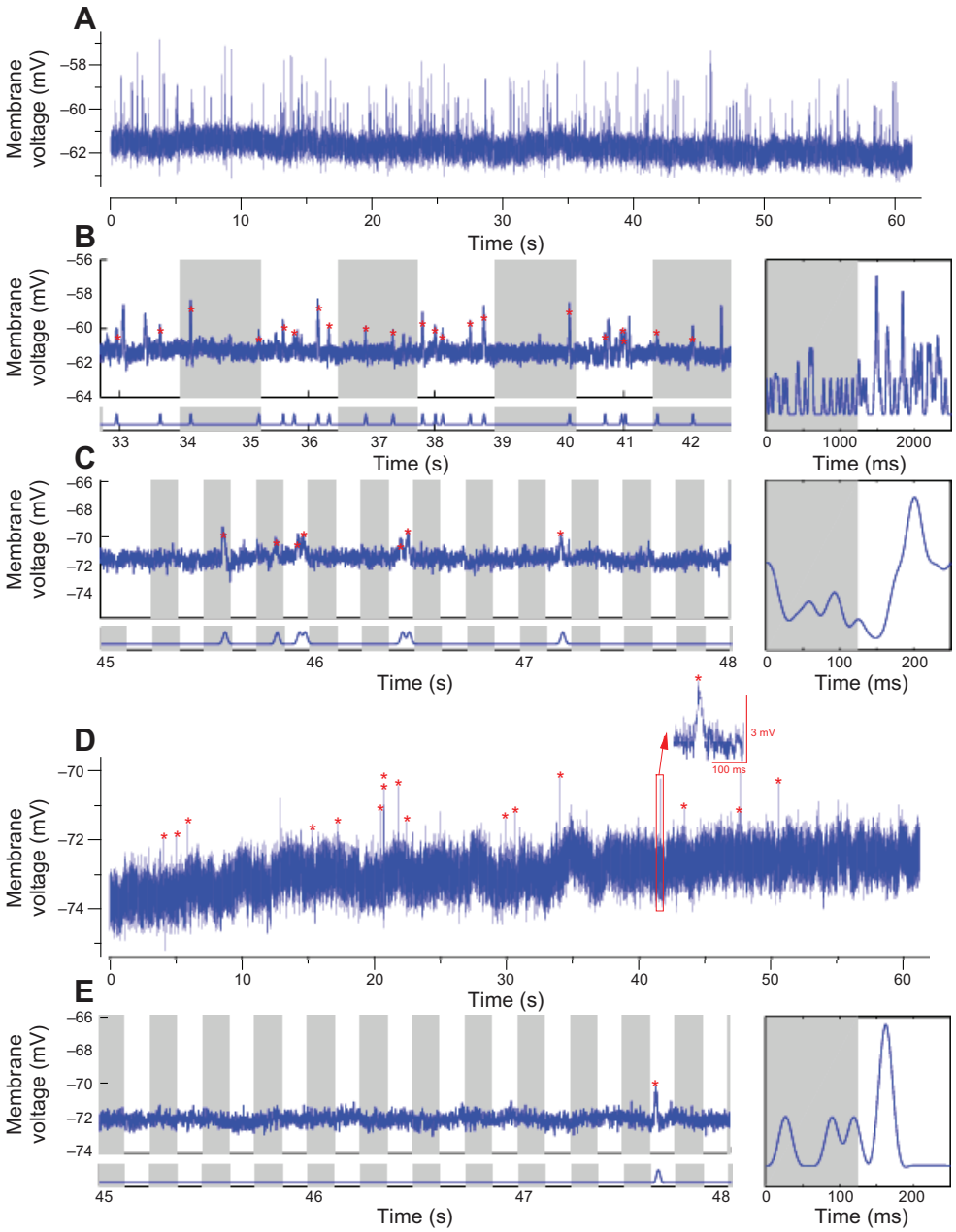

Fig. 5. Examples of photoreceptor responses near the behavioural threshold. Quantum bumps at 0.05 lx (A–C) and 0.005 lx (D,E). Grey-and-white background denotes the blacks and whites of the rotating grating passing the visual field of the photoreceptor. (A) A recording with the 0.4 Hz stimulus shows bumps during control (0–30 s) and rotation (30–60 s). (B) A 10 s subset of A, with red asterisks denoting identified bumps, and template bumps produced beneath. Cumulated bump sums during successive 2500 ms stimulus cycles are shown on the right (12 cycles per 30 s); bump rate is elevated during the latter 1250 ms when the cell faces white. (C) A 3 s excerpt of a recording with a 4 Hz stimulus. The right-hand panel shows stronger bump synchronization with white than that in B. (D) A recording at 0.005 lx showing characteristically few bumps. The inset image shows the typical bump waveform. (E) A 3 s example with a 4 Hz stimulus (left) contains only one bump. A scarcity of photons leads to similar bump sums for black and white (right). Significant optomotor responses were still measured under these conditions.

similar to each other, as were the behavioural responses at these light intensities (Fig. 2A,B).

At the lowest light intensities of 0.05 and 0.005 lx, all photoreceptor responses to control and rotating stimuli consisted only of separate single-photon responses, quantum bumps (Fig. 5A,D). The number of bumps during rotation was ca. 1 s^{-1} at 0.05 lx and 0.1 s^{-1} at 0.005 lx (Table 1). In some cells, bump occurrence was clearly synchronized with the temporal period of the stimulus at low frequencies of 0.1–4 Hz (Fig. 5B). In a subset of cells, the 30 s stimulus presentation with low temporal frequencies was sufficient to obtain a large enough number of single photon signals to observe their synchronization with the stimulus: a larger sum of bumps occurred during the presentation of the white bar (Fig. 5B,C, right-hand panels). Larger bump sums on white could not be distinguished with 0.005 lx intensity (Fig. 5E). Some cells were tested with a 10-fold dimmer stimulus at 0.0005 lx, but no bumps were generated during the entire 60 s recording.

DISCUSSION

The results of intracellular recordings around the behavioural threshold of 0.005 lx showed the quantal nature of cockroach photoreceptor responses. Strikingly, at the lowest intensity where the optomotor response was present, at 0.005 lx, the stimulus elicited only approximately one single photon response in 10 s (~0.1 photons s^{-1}) per photoreceptor (Table 1). Previously, Dubs et al. (Dubs et al., 1981) have used a uniform screen of the same mean luminance, instead of the moving optomotor grating, in determining a mean bump rate at the behavioural threshold in the housefly *Musca domestica*, which was 1.7±0.7 s^{-1}. For the cockroach, the threshold is approximately 20 times lower, approximately 0.1 photons s^{-1}.

The shade response of *P. americana* persists unattenuated down to at least 0.01 lx (Okada and Toh, 1998), which agrees well with our results. In our experiments with an even lower intensity of 0.005 lx, responses between 0.4 and 4 Hz were still significant but

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Table 1. The average number of bumps s^{-1} at 0.05 and 0.005 lx

Frequency (Hz)	0.05 lx			0.005 lx		
	Mean	s.d.	*n*	Mean	s.d.	*n*
0.1	0.96	0.88	7	0.10	0.20	8
0.4	0.66	0.91	5	0.14	0.23	7
2.4	1.1	0.86	6	0.11	0.13	8
4	1.1	0.87	6	0.14	0.18	7
6	1.0	1.0	5	0.11	0.13	8
10	0.90	0.95	6	0.18	0.21	6
12	0.96	1.1	5	0.085	0.11	8
18	0.89	1.0	5	0.13	0.13	7
Total	0.95	0.90	7	0.12	0.16	9

Frequency, temporal frequency of the rotating stimulus; mean, mean number of bumps s^{-1}; *n*, number of cells analyzed.

weaker than those at brighter intensities. The optomotor response in the nocturnal mosquito *Anopheles gambiae* remains in white light down to 10^{-5} W m^{-2} (Gibson, 1995), which equates to 0.005 lx used in this study. The mean light intensity in the VR setup we used is even lower, attributable to the 50% black and 50% white stimulus grating that was used. Visual behaviour may be possible at even lower average intensities, as shown in studies with nocturnal bees foraging in their natural habitats: *Xylocopa tranquebarica* at 10^{-5} cd m^{-2} (Somanathan et al., 2008) (corresponding in our estimation to ~6.3×10^{-5} lx) and *Megalopta genalis* at 10^{-4} cd m^{-2} (Warrant et al., 2004) (corresponding to ~6.3×10^{-4} lx); and foraging toads in the laboratory settings at 10^{-5} lx (Aho et al., 1988). However, natural forest environments and the experimental setup used by Aho et al. (Aho et al., 1988) probably contain higher local contrasts than the 0.33 that is attainable in the VR, possibly making movement detection more challenging in the latter (Heimonen et al., 2012; O'Carroll et al., 2012).

The discrete and random nature of photoreceptor signals (Fig. 5), combined with the results of the optomotor experiments (Fig. 2), indicate that massive temporal and spatial pooling takes place in higher order neuropiles for computing the motion from the photoreceptor signals in the dark. This probably takes place in the hypothetical elementary movement detectors (EMDs) (for example, see Borst et al., 2010), as suggested earlier for the diurnal house-fly (*Musca domestica*) (Pick and Buchner, 1979). Already at the photoreceptor level, the angular sensitivity in the cockroach increases in dark adaptation (Butler and Horridge, 1973) to reach an average value of 6.4 deg (Heimonen et al., 2006). In terms of anatomy, the adult cockroach has 26,000–45,000 photoreceptors in each eye and approximately 3000 third-order neurons in the medulla (Füller et al., 1989), and the estimated number of second-order neurons is 2000–3000 (Heimonen et al., 2006). This indicates pooling (here: spatial summation) of 6–20 photoreceptors to one second-order cell at the first stage of processing (Ribi, 1977). However, in darkness, more spatial summation is generally required at higher stages to create a signal, with which a movement detector could operate (Borst et al., 2010; Egelhaaf et al., 2012). In nocturnal bees the optimal spatial summation, both with high stimulus velocities and low light intensities, requires a recruitment of 12 to 30 s order units (lamina cartridges) at the lowest intensity tested (Theobald et al., 2006). Although the cockroach lamina is not arranged in discrete cartridges (Ernst and Füller, 1987; Ribi, 1977), similar branching of photoreceptor axons and L-fibres has been found both in the cockroach and the nocturnal bee (Greiner et al., 2004; Ribi, 1977). The evidence of increasing spatial summation at lower light intensities (Fig. 2E–H) allows us to estimate the extent of the summation in the cockroach motion detection system. Because the smallest interommatidial angles in the central part of cockroach eye are around 1 deg (Butler, 1973), then a circular area 90 deg in diameter (corresponding to the angular period of the smallest effective stimulus at 0.005 lx) would involve ~6400 ommatidia and ~30,000 green-sensitive receptors (Butler, 1971), more than there actually exists in the eye. However, a more realistic estimate would be to use the half-width of the angular period, assuming that the occurrence of light–dark borders (i.e. with a 45 deg period) is a meaningful measure. This would give the corresponding numbers of ~1600 ommatidia and ~8000 photoreceptors. With a 4 Hz stimulus at 0.005 lx, each receptor received 0.1 photons s^{-1}, resulting in 0.025 photons $cycle^{-1}$. This leads to ~200 photons in the calculated area $cycle^{-1}$, giving a maximum signal to noise ratio (SNR) for a shot-noise-only limited signal of approximately 14. It has to be emphasized that the number of pooled photoreceptor signals here is estimated, not on the basis of the SNR needed, but on the basis of the experimental results. The true SNR of pooled signals is likely to be significantly lower than 14, because of the semi-chaotic organization of the cockroach retina and the poor general performance of the photoreceptors (Heimonen et al., 2006). It is also very likely that other noise sources on the pathway from photoreceptors to the summing units, e.g. the EMDs, will render the SNR submaximal. If movement were seen by the peripheral, more curved areas of the eye, the number of pooled photoreceptors would be smaller, with a corresponding lowering of the SNR estimate.

The optomotor response was similar at 500 and 5 lx, and only a little attenuated at 0.05 lx, supporting the idea that cockroach photoreceptors are optimized to low light intensities (Heimonen et al., 2006; Heimonen et al., 2012) and that all significant adaptive changes take place below the light intensity of approximately 1000 photons s^{-1}. The nearly constant response at high intensities also indicates that spatial summation takes place at all ecologically relevant intensities (Theobald et al., 2006). The cockroach has 16,000–28,000 green-sensitive photoreceptors per eye (Butler, 1971; Füller et al., 1989), and our estimates suggest that signals from hundreds to thousands of receptors are pooled at the intensity of 0.005 lx. This is really massive pooling. In the vertebrate eye, the rod signals are pooled massively in the dark, e.g. in the cat 1500:1 (Sterling et al., 1988), but compared with the total amount of photoreceptors available, the cockroach case is of a different magnitude altogether. We can also surmise that one of the reasons for big eyes in nocturnal animals may thus be the need for a large number of receptors for efficient pooling when very few photons are available. The massive pooling of signals that is necessary in the cockroach visual system for motion detection has to rely on unknown neural processing in the deeper ganglia, in order to cope with the inescapably deteriorating spatial resolution. The study of those processing mechanisms is likely to be very fruitful for designing bio-mimetic imaging systems for night vision.

MATERIALS AND METHODS

Animals

We used adult male wild-type *Periplaneta americana* from a laboratory colony. The individuals were removed from the colony after their final ecdysis to prevent any physical damage caused by other individuals and were used in experiments within three months of ecdysis. Animals were kept at 25–27°C under 12:12 h light–dark regime with food and water available *ad libitum*. Experiments were performed mainly during the dark phase of the 12:12 rhythm, but occasionally they continued into the light phase. Results obtained during light and dark were not significantly different from each other.

Behavioural experiments

We used a VR setup, described previously (Takalo et al., 2012), in the open-loop mode. The stimulus was a grating of vertical black and white bars with

angular periods between 20 deg and 180 deg. The stimulus grating rotated at nine different speeds producing nine temporal frequencies: 0.1, 0.4, 2.4, 4, 6, 10, 12, 15 and 18 Hz (supplementary material Movie 1). For testing the responses with different temporal frequencies, the angular period of 60 deg was chosen. The unattenuated light intensity of the optomotor stimulus pattern from the projector (DepthQ®, Lightspeed Design, Bellevue, Washington, USA) reflecting *via* the projection surface, as measured in the centre of the sphere, was 500 lx. The spectrum of a full-field image at 100% brightness peaks at approximately 440, 550 and 575 nm (Takalo et al., 2012). Lower light intensities were created by placing −2, −4, −5 and −6 decade neutral density filters (NE Series, Thorlabs, Newton, New Jersey, USA) in front of the projector lens. The Michelson contrast of the stimulus was 0.33 at all light levels.

Experiments were performed at room temperature (ca. 20°C). For capture and preparation, the cockroach was anaesthetized with CO_2, and the tip of a metal-wire holder was attached to its pronotum with a mixture of beeswax, paraffin and resin. The animal was placed on the trackball so that it had a 135 deg lateral view of the projection surface on both its right and left side. The placement was such that it allowed the cockroach to move its head, legs and abdomen, but that it could not touch the ball with its mouthparts (Fig. 1A,B). Antennal contact with the curved ball surface was infrequent, and the absence of tactile and chemosensory cues motivated the animal to walk for hours regardless of its diurnal activity phase. Cockroaches were allowed to fully recover from the anaesthesia and assume normal walking, running and antennal movements before any experiments began. They were dark-adapted (i.e. no images were projected onto the VR sphere) for 15–20 min every time a lower light level was used. Stimulus sequences consisted of an initial 60 s control period, during which the stimulus pattern was presented but did not move, followed by a 30 s rotation of the stimulus in a randomly chosen direction (clockwise or anticlockwise), another 30 s of control, and finally, a 30 s period of rotation to the opposite direction from that of the first rotation. Rotation in both the clockwise and anticlockwise direction during each trial removed from the data any confounding factors caused by the possible sidedness or side preference of the animal and minor deviations from the correct alignment of the animal on the trackball. The two rotational directions yielded two different response strength values (see Data analysis and bump counting) of rotation and control for each cockroach. Between trials, all lights inside the virtual reality sphere were off.

Intracellular recordings

For intracellular recordings, nine of the cockroaches that had been used in the behavioural experiments were anaesthetized and prepared as described previously (Heimonen et al., 2006). The preparation included the head and the thorax of the animal. The head was tilted to the right and fixed in this position with wax. Antennae were removed and facial muscles cut to prevent damage to the electrode tip. A rectangular hole for the recording electrode was made near the dorsocaudal margin of the left eye, and an opening for the reference electrode was cut into the pronotum (Fig. 1C). Borosilicate glass microelectrodes (resistance 80–120 MΩ) were made with a laser micropipette puller P-2000 (Sutter Instrument, Novato, California, USA) and filled with 2 M KCl. The cockroach preparation was placed onto a holder in the centre of the VR sphere. The chloride-coated silver wire acting as a reference electrode was introduced into the pronotum. The preparation was slightly rotated around the vertical axis of the holder so that the micromanipulator (SMXS-system, Sensapex, Oulu, Finland) could bring the recording electrode into contact with the retina under visual control *via* a stereomicroscope (Nikon SMZ660, Tokyo, Japan). The recording electrode was introduced into the retina from behind with respect to the direction in which most of the eye was pointing. After photoreceptor impalement, the directions of their optical axes were determined at the brightest available light level (intensity 500 lx). Capacitance compensation and signal amplification were made using an intracellular amplifier (NPI SEC-05L, Tamm, Germany). The stationary and movement stimuli had an angular period of 60 deg and were otherwise identical to those used in the behavioural experiments, except for their total duration and unidirectionality of their rotation. The stimulus sequence was: 30 s control and 30 s rotation. Photoreceptor recordings were only accepted for further analysis if they were sufficiently stable and met basic electrophysiological quality criteria (resting potential, maximum light response) that has been defined previously (Heimonen et al., 2006). When continuing to lower light levels, the preparations were dark adapted for ca. 15 min in complete darkness.

Data analysis and bump counting

The behavioural data were analyzed using MATLAB (MathWorks, Natick, Massachusetts, USA), and statistical tests were run with OriginPro 8.6 (OriginLab Corporation, Northampton, Massachusetts, USA). The total angular distance covered by the cockroach during the rotating stimulus or control ($Angle_{total}$) was readable from the data, as well as the angular distance moved in the direction of the stimulus ($Angle_{follow}$). The strength of the behavioural response was calculated as:

$$\text{Response strength} = 2(\text{Angle}_{\text{follow}} / \text{Angle}_{\text{total}}) - 1,$$

where $Angle_{total}=Angle_{follow}+Angle_{wrong}$. Response strengths for the stationary control patterns were calculated as if the stimulus pattern were moving to the direction of the rotation following that control. The response strength was calculated separately for each direction of rotation, so that each experiment with a certain stimulus frequency yielded two measures of the response strength.

In an open-loop experiment, cockroaches follow the direction, but not the rotation velocity, of the moving stimulus. Supplementary material Fig. S1 illustrates the independence of the stimulus velocity and the cockroach walking velocity. Therefore, with the American cockroach, the strength of the stimulus-following response cannot be calculated as $Angle_{follow}$ divided by the angular distance moved by the stimulus pattern. Moreover, stopping during movement is quite normal cockroach behaviour and can happen during the experiments (Okada and Toh, 2004). Video recordings of experiments (supplementary material Movie 1) show that the animals could stop even when the rotating stimulus is showing. Although they were standing still during the stop, they still moved their antennae to the direction of the stimulus motion if the stimulus was one that they would have been following when walking. Angular distances, i.e. comparing the following performance of each cockroach against its own movement activity, were found to be the best measure of the response strength even when the locomotor activity was interspersed with random stops. The angular distances were a reliable measure if the total angular distance moved during a trial was more than 360 deg. Typically, cockroaches walked many times further than this during a 30 s trial.

Intracellular signals were analyzed with custom software made in the MATLAB (MathWorks, USA) environment. Determinations of single-photon responses (bump counting) were performed by first band-pass filtering the voltage recordings, in order to remove unwanted low-frequency trends and to reduce the high-frequency noise. Subsequently, a threshold was set for finding the events that were unmistakably quantum bumps – these were averaged to produce a recording-specific bump template. The template was fitted point by point to the entire recording with a minimum amplitude criterion and with the acceptance of 'bumps' based on a mean squared error estimate. The bump counting was possible with the methods used because our earlier findings showed that the amplitudes and durations of quantum bumps formed a continuous distribution, indicating that only one class of event is present (Immonen et al., 2014), in contrast to findings in a few other insect species, such as flies (Dubs et al., 1981), locusts (Lillywhite, 1978) and crickets (Frolov et al., 2014).

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Competing interests

The authors declare no competing financial interests.

Author contributions

A.H., K.H., M.V. and M.W. designed the experiments. A.H. performed the experiments and analyzed the data. J.T. wrote the software used in the experiments and data analyses, made the stimuli and contributed to the data analyses. A.H., M.V. and M.W. wrote the manuscript.

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Supplementary material

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