Signal coding in cockroach photoreceptors is tuned to dim environments

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SENSORY SYSTEMS PROVIDE ANIMALS with necessary information for survival and reproduction. Like all senses of different species, visual systems are thought to have selectively adapted for functioning under their prevailing environmental conditions during their evolution and development (Laughlin 1989, 1990, 1996; Warrant 1999, 2004; Weckström and Laughlin 1995). However, for species living in dim environments, extracting visual information can be a self-contradictory task, because there may be little reliable information to be gathered within behaviorally relevant integration times. With only a few photons to absorb, vision becomes unreliable (Warrant 1999, 2006). The random (Poisson-distributed) arrival of photons triggers single-photon responses, so-called quantum bumps, in the photoreceptors, generating photon shot noise and inevitably lowering the signal-to-noise ratio (SNR). The macroscopic photoreceptor responses are formed when the bumps are integrated by the nontransductive part of the photoreceptor membrane (Wong and Knight 1980; Wong et al. 1980). An intrinsic but single-photon-related source of noise, called transducer noise, varies timing, shapes, and sizes of the bumps, further lowering the SNR of the macroscopic response (Laughlin and Lillywhite 1982; Lillywhite and Laughlin 1979). Dark noise (spontaneous bumps without light), which may be significant in vertebrate vision (Aho et al. 1988), is of minor importance in invertebrates (Heimonen and Weckström, unpublished observations; Laughlin and Lillywhite 1982; Lillywhite 1977) and likely to be suppressed specifically by a molecular mechanism within the phototransduction cascade (Katzen and Minke 2012).

Vision generally improves with increasing brightness. Light adaptation (LA) makes phototransduction faster and quantum bumps smaller and their latencies shorter (Howard et al. 1987; Juusola et al. 1994). Concomitantly, transduction gain (response amplitude/unit of stimulus intensity) decreases, although contrast gain (response amplitude/unit of stimulus contrast) increases. Such signaling dynamics have been described in detail in several insects (Fairire and Juusola 2008; Juusola and Hardie 2001; Juusola et al. 1994; Niven and Laughlin 2008; Niven et al. 2003). With LA, noise becomes less significant, and the SNR of responses improves. In dim conditions, the only way for the visual system to make sense of the visual environment is to increase sensitivity by integrating more signals over space and time (Laughlin 1990; Warrant 1999, 2006, 2008; Warrant and McIntyre 1993). This includes light collection from wider receptive fields, pooling of signals from several photoreceptors, and longer integration times of the photoreceptors. At the level of photoreceptors, there are some apparent general strategies to cope with the problem of photon scarcity: high gain of phototransduction and temporal and spatial summation.

Good vision in dim light is achieved by large-sized camera eyes or in insects, generally by superposition compound eyes with specialized features for efficient gathering of light (Laughlin 1990; Warrant 1999, 2004). In contrast, apposition compound eyes, such as those in the cockroach (Butler 1971, 1973b), may be thought to be inadequate for night vision. However, cockroaches are active mainly under dim conditions (Guthrie and Tindall 1968; Lipton and Sutherland 1970; Roberts 1965). They tend to avoid light when given a choice (Guthrie and Tindall 1968; Halloy et al. 2007; Kelly and Mote 1990), directing their escape responses toward dark places (Riemay 1984; Ye et al. 2003). In addition, there are other nocturnal...
species, which have apposition eyes [see review by Warrant (2008)]. Contrary to compound eyes in general, cockroach eyes have a highly irregular structure (Butler 1973a, 1973b; Ernst and Füller 1987; Ribi 1977). Functionally, they show variable responsiveness of the photoreceptors (Heimonen et al. 2006) and spike coding in the photoreceptor axons (Weckström et al. 1993).

In the present work, we explore the response dynamics of cockroach photoreceptors to investigate whether they are selectively adapted for operating in dim light and whether cockroaches differ in this respect from other species studied with similar techniques. The results, obtained by intracellular recordings of intact photoreceptors and patch-clamping of isolated ommatidia and with the use of both white noise (WN)-modulated and naturalistic stimulation, reveal exclusive physiological adaptations of cockroach photoreceptors to dark and emphasize the value of naturalistic stimulation as a tool of choice for studying early visual encoding.

METHODS

Animals and preparation. Recordings were performed from green-sensitive photoreceptors of adult male cockroaches (Periplaneta americana). Some females were also tested to ensure that their voltage responses to light stimuli did not differ significantly from those of males. Cockroaches were maintained for all of the experiments and prepared for the in vivo intracellular experiments as described in Heimonen et al. (2006). After preparing the animal and positioning the recording and indifferent electrodes, the preparation was dark adapted for ~30 min. All intracellular recordings were done in the retina, where the somata of the photoreceptors are located. Under a stereomicroscope, the retina of the cockroach is seen below the cornea as a black, highly pigmented area, and the location of recording electrode in the preparation could be identified on these grounds. This location is also characterized by graded-depolarizing responses, which lack the superimposed spikes typical for cockroach photoreceptor axon recordings (Heimonen et al. 2006; Weckström et al. 1993). No attempts were made to distinguish between the responses of the short- and long-type photoreceptors, which terminate in the first and second neuropile, respectively (Ribi 1977). For the in vitro whole-cell patch-clamp experiments on dissociated cockroach ommatidia, including the somata of the eight photoreceptors, the preparation was done similarly as described for Drosophila melanogaster (Hardie 1991). Shortly after dissection in Ringer’s solution, both retinae were cut into piece small enough to barely fit into a glass capillary, fire polished to an opening diameter of ~500 μm. Subsequently, the retina pieces were transferred into a drop of Ringer’s solution supplemented with 0.2 mg/ml collagenase type 2 (Worthington Biochemical, Lakewood, NJ) and 0.2 mg/ml pankreatin (Sigma-Aldrich, St. Louis, MO) for 10 min incubation before the actual dissociation. The dissociation of ommatidia was done by triturating the enzymatically treated pieces with a series of three to four fire-polished glass capillaries (from 500 to 250 μm opening diameter). The dissociated ommatidia were then transferred into the recording chamber and allowed to settle in darkness for a few minutes.

Setup for in vivo experiments. Intracellular voltage responses of photoreceptors to light or current stimulation were recorded at room temperature (~20°C) with borosilicate glass capillary microelectrodes (resistances 50–150 MΩ), filled with a 2-M KCl solution, whose pH was adjusted to 6.84 with a K-phosphate buffer. The electrode capacitance was compensated and the signals amplified with an intracellular amplifier (SEC-05L; NPI, Germany). The light stimuli were produced with a green, high-intensity light-emitting diode (LED; Roithner LaserTechnik, Austria; peak wavelength, 525 nm). The computer-generated stimulus waveforms for the LED were fed to a custom-made voltage-to-current driver, which controlled the current and thus the intensity modulation of the LED output. The driver was equipped with a voltage output for monitoring the LED current. Additional background intensity control (7 log units) was achieved by placing neutral density filters (Kodak Wratten, Los Angeles, CA) between the stimulating light and the eye. A Cardan arm system allowed the LED to be moved along the surface of an imaginary sphere (radius ~50 mm) with the eye in the center. In this configuration, the LED spatially subtended ~2° at the photoreceptor level. The current stimulus waveforms were produced and injected into the cells through the amplifier and the recording electrode (in current-clamp mode, using ~8 kHz switching frequency). The stimulus waveforms were produced, the measurements controlled, and the signals sampled and analyzed with a Matlab (MathWorks, Natick, MA)-based, custom-written software (M. Juusola), running on a computer equipped with a data acquisition card (National Instruments, Austin, TX; PCI-MIO-16E-4). The monitored stimuli and the amplified responses were low-pass filtered at 250, 500, or 700 Hz (Kemo VBF/23) and sampled at 0.5, 1.0, or 1.2 kHz.

Setup for in vitro experiments. The whole-cell patch-clamp recordings were performed at room temperature (~20°C) using borosilicate microelectrodes pulled to have a resistance of 3–8 MΩ. The electrodes were filled with a solution containing (in mM) 140 KCl, 0.4 MgCl₂, 4 Mg ATP, 0.4 Na GTP, and 1 NAD (pH adjusted to 7.15 with potassium hydroxide). The cockroach Ringer (extracellular solution) contained (in mM) 120 NaCl, 5 KCl, 4 MgCl₂, 1.5 CaCl₂, 10 TES, 25 proline, and 5 alanine (pH adjusted to 7.15 with sodium hydroxide). The liquid junction potential between the solutions (~4 mV) was considered negligible for these recordings. Signals were amplified with Axopatch one-dimensional amplifier (Molecular Devices, Sunnyvale, CA), filtered with an eight-pole Bessel filter, and recorded, sampled, and analyzed with pCLAMP 9 software (Molecular Devices). The light stimulation was provided by a green LED (525 nm), driven by a similar system as described above. The recordings were sampled at 2 kHz and low-pass filtered at 1 kHz.

Stimulus protocols. In the in vivo experiments, all light stimuli were given along the optical axis of each photoreceptor. This axis was localized before actual recordings by finding the maximal response to a test light flash when moving the light source across the receptive field of each cell. In the in vitro experiments, the light stimuli were given to isolated ommatidia in a bath solution through the microscopic optics. For each photoreceptor, the intensity of the stimulating LED was first calibrated by counting the number of single-photon responses (quantum bumps) during constant dim illumination (background light). This gave a cell-specific relative measure of intensity in so-called effective photons/s (Heimonen et al. 2006; Juusola et al. 1994). Thereafter, the light stimulus waveforms were chosen according to the experiment in question, the cell’s functional category (Heimonen et al. 2006), and the need for additional results. The used waveforms were pulses with different intensities, contrasts, and durations or pseudorandomly modulated contrast sequences (filtered Gaussian WN), both at different intensities of background light (a more complete account of these stimuli is reported in Juusola 1993, 1994; Kouvalainen et al. 1994). Alternatively, the waveforms could be a time series of naturalistic light-intensity variation [naturalistic intensity series (NIS)] selected from van Hateren’s (1997) natural image database. When LA responses were recorded, the originally dark-adapted (DA) photoreceptors were exposed to each background light level for at least 90 s before introducing contrast stimuli (steps, WN, or NIS). This aimed to ensure that the sensitivity of the photoreceptors had reached a steady-state and that most adaptation processes were completed, including possible palisade formation and pigment migration (Butler 1971, 1973b; Ferrell and Reitcheck 1993; Snyder and Horridge 1972). Long periods of light stimulation, especially with bright intensities, were always followed by DA for several minutes. The length of DA was considered to be adequate when the response to a test flash had attained its original amplitude and overall shape. The light contrast (c) was defined as the change in light intensity (ΔI)
divided by the mean light background ($I$). In the case of pseudorandom contrast modulation, the SD of the stimulus intensity was taken as $\Delta I$. The SD of WN contrast modulation was 0.32. The experiments were performed first at the lowest level of background light before proceeding to the brighter ones. Additionally, to investigate the properties of the photoinsensitive cell membrane, current pulses or pseudorandom WN current sequences were injected through the recording electrode into the photoreceptors in the discontinuous current-clamp (switched-clamp) mode of the amplifier. The SD of the pseudorandom current was 0.16 nA.

Quality of recordings. In vivo intracellular recordings had to fulfill the following quality requirements to be included in this study: $-65$ mV < resting potential < $-55$ mV; input resistance $\geq 60$ M$\Omega$ (tested with a hyperpolarizing $-0.3$-nA current pulse at the dark resting potential), and they have clearly visible quantum bumps with amplitudes of several millivolts (Fig. 1) at low-light intensities. Typically, the resting potential was $\approx -60$ mV and the input resistance 70–80 M$\Omega$, occasionally even 100 M$\Omega$. Recordings were also rejected if after a period of light stimulation, the sensitivities and time courses of test flash responses failed to return to their initial values within minutes of DA. In vitro whole-cell recordings had to fulfill the following criteria to be selected into this study: resting potential $\approx -50$ mV (recorded under current clamp in $I = 0$ mode of the amplifier), series resistance $< 30$ M$\Omega$ before compensations, and input resistance $> 500$ M$\Omega$ (tested under voltage clamp below rest with a voltage pulse from $-70$ to $-80$ mV). The resting potential was, on average, $-59 \pm 6$ mV ($\pm$SD; $n = 20$), the series resistance $< 5$ M$\Omega$ after compensation, and the input resistance always $> 1$ G$\Omega$.

In vivo intracellular recordings. The frequency response (presented as the gain and the phase functions) and the coherence function between the WN-modulated contrast stimulus and the induced photoreceptor voltage responses (French 1980a, b), as well as the SNR of the responses in frequency domain ($SNR(f)$), were all recorded and computed as reported earlier (Juusola et al. 1994; Kouvalainen et al. 1994). Typically, 16-s-long data sequences sampled at 1 kHz were used. The frequency domain functions, including membrane impedance (frequency response between injected current and recorded membrane voltage), were calculated as averages of 10–20 adjacent responses to a pseudorandom stimulus sequence. The linear-impulse responses (first-order Wiener kernels) were obtained from the frequency responses via the inverse fast Fourier transform. Details of how to measure cell impedances in vivo, using WN-modulated current injections, are reported earlier (Juusola and Weckström 1993; Weckström et al. 1992). The coherence functions between NIS and photoreceptor voltage responses and the $SNR(f)$ of these responses were also recorded and analyzed as described earlier (Heimonen et al. 2006; Juusola et al. 1994; Kouvalainen et al. 1994). Here, 10 10-s-long data sequences were sampled at 1.2 kHz and averaged. Finally, the signaling performance of cockroach photoreceptors was quantified (in bits/s) from the voltage responses to a 100–200× repeated NIS, using the triple extrapolation method (Juusola and de Polavieja 2003). This method measures the rate of information transfer as the differences between the response entropy and the noise entropy rates. Here, the duration of the recorded sequences was 2 s, the sampling frequency 500 Hz, and both stimuli and responses were low-pass filtered at 250 Hz.

In vitro whole-cell recordings. Responses to bright, 10-s-long light pulses were recorded both in $I = 0$ and voltage-clamp mode (at a holding potential of $-70$ mV) of the amplifier in the same cells to justify direct comparison of their waveforms. SNR of responses
because of their large amplitude. The initial peak transient (the first second of the response) was left out of the analysis, and the rest of the responses (1–10 s) was detrended by using a sixth-order polynomial fit. The SNR \( f \) was first analyzed as reported previously (Kouvalainen et al. 1994). Then, in SNR time domain \( [\text{SNR}(t)] \), was estimated in the following way: 1) the detrended responses were divided into 10 equal length-time segments (10; 0.9-s bins); 2) the corresponding response segments were averaged to give an estimated signal; and 3) the variance of this signal was divided by that of noise (the difference between the signal and an original detrended response) to obtain \( \text{SNR}(t) \) of each segment. In the SNR recordings, the mean light intensity was always bright enough to produce saturated responses in DA cells.

**RESULTS**

The most remarkable and unusual feature in cockroach photoreceptor function is the large variability in their voltage responses (Heimonen et al. 2006). This functional diversity, which seems not to follow any clear retinotopical organization, can be used to categorize the photoreceptors according to their adaptation profile, or rate of light-response decay, during light stimulation (Fig. 1). The three photoreceptor categories: “hyperadapting”, “adapting”, and “nonadapting” typify two extremes and an intermediate behavior of an actually continuous distribution of response variability (Heimonen et al. 2006).

In the present study, we examined how this functional diversity affects visual encoding. We first present results from in vivo (intracellular) and in vitro (whole-cell patch-clamp) recordings in DA photoreceptors. The light-induced current (LIC) responses (Fig. 2) were examined to find out whether the source of the variability of the light-voltage responses is in the LIC. Then, we examined how LA photoreceptors can code contrast steps into voltage responses (Fig. 3) at various light levels and if this shows functional specialization to dark. The dynamics of contrast coding was investigated further by recording and analyzing responses to WN-modulated contrast sequences at different adapting backgrounds (Fig. 4). The electrical properties of the light-insensitive photoreceptor membrane were quantified to examine their contribution to coding (Fig. 5). Finally, to determine the photoreceptor performance at various light levels, we measured the SNR, both the \( \text{SNR}(f) \) and the \( \text{SNR}(t) \), of voltage responses after the light onset (Fig. 6) and computed the rate of information transfer for NIS (Fig. 7). These results quantify how the response properties of cockroach photoreceptors are selectively adapted for encoding visual signals in scotopic environments.

**Light responses of DA photoreceptors.** The DA voltage responses were recorded first to show the variability as described before (Heimonen et al. 2006) and secondly, to further study the LA as it is reflected in response shapes with long light pulses of up to 10 s. When dark adapted, the impulse responses of different types of photoreceptors were often similar. However, the cells could be distinguished by their ~100-fold sensitivity differences, as determined by their individual voltage vs. log of intensity (V-logI) curves (Heimonen et al. 2006; Weckström et al. 1993). With longer light pulses, differences in adaptation emerged (Figs. 1, A and C).

Whereas the early transient responses of different cells (Fig. 1C) had similar shapes, they repolarized differently during constant light. Cells that were categorized earlier as nonadapting, using 300 ms stimuli (Heimonen et al. 2006), actually responded with slow repolarization toward dark resting potential when longer, 10-s light pulses were used (Fig. 1). Hence, they are better termed “slowly adapting”. Fig. 1 also shows responses of the hyperadapting cells. Surprisingly, these photoreceptors produced bump-like events with more or less unattenuated amplitudes in bright light when the initial step response had repolarized close to the dark resting potential (Fig. 1B). Thus the “hyperadaptation” is more like reduced excitation efficiency than adaptation of bumps. Although here

![Current clamp](http://jn.physiology.org/figure10_12.png) ![Voltage clamp](http://jn.physiology.org/figure10_13.png)

Fig. 2. In vitro whole-cell patch-clamp recordings of DA photoreceptors show similar variability both in voltage responses (top row) and in light-induced current (LICs; bottom row; at −70 mV) when stimulated with bright (saturating), 10-s-long light pulses (timing shown with bars between the response rows). The responses in each column are produced by the same (repeated) stimulus and recorded from the same cell. The time courses of the voltage responses are closely mirroring the shape and waveform of the corresponding LICs. Scales are the same for all of the responses. The transient early responses of the LICs are clipped because of their large amplitude.
found to be a misnomer, the hyperadapting shall be used in the following to be consistent with the existing nomenclature (Heimonen et al. 2006) when referring to those kinds of photoreceptor responses.

In very dim conditions, the light responses of all cockroach photoreceptors consisted only of single-photon-induced discrete events—quantum bumps (Fig. 1B). At stimulus intensities of <10 photons/s, the bumps were large (up to 5–10 mV). In contrast to the ~100-fold variation in sensitivity of graded light responses (Heimonen et al. 2006; Weckström et al. 1993), the differences in the absolute sensitivity were nearly 10 times less. With the same dim background, the average bump frequency of different cells varied between two and 20 bumps/s (9 ± 4 bumps/s; mean ± SD; n = 18).

The findings thus showed that cockroach photoreceptors can generate large voltage responses to single photons and that the macroscopic responses tend to adapt toward the resting potential.

**LICs and corresponding voltage responses.** To search for the sources of functional variability, we recorded LICs from isolated photoreceptors in voltage-clamp mode (Fig. 2; note that the large, unreliable, initial >6-nA transients are truncated). When comparing voltage and current step responses for saturating responses, we found that their waveform dynamics were nearly equivalent in each of the cells. For example, the voltage response of a hyperadapting cell closely followed the time course of its LIC. These results suggest that 1) the variability in voltage responses reflects the variability in the phototransduction process (LIC) and that 2) the role of the nontransductive membrane properties in shaping the response waveforms is limited compared with the LIC.

**Contrast coding with step responses in time domain.** We next examined how the different cells encoded light contrasts at different adapting backgrounds. Photoreceptors were first light adapted to different light levels and then stimulated with contrast pulses, i.e., light increments or decrements (Fig. 3A). In response to a prolonged (~90 s) constant light exposure and adaptation, surprisingly, only 39% of the cells (nine of 23) sustained their steady-state depolarization markedly (5–15 mV) above the dark resting potential (Figs. 3, A and B). In these photoreceptors, originally categorized either adapting or non/slowly adapting, the contrast gain (the slope and range of their V-logI curves) increased marginally with increasing light intensity. In other words, the same contrasts evoked larger responses at brighter backgrounds. In particular, we found that the higher the maintained steady-state depolarization, the larger the contrast-evoked responses. However, this increase in contrast gain took place only at low background intensities (Fig. 3B). When the steady-state depolarization saturated, the contrast-evoked responses saturated as well. This usually occurred at an intensity range of 1,000–3,000 photons/s (eight of nine cells) and in only one case at ~10,000 photons/s. The responses to light increments and decrements were only roughly symmetrical, with the smallest (0.2–0.4) contrasts reminiscent of those in fly photoreceptors (Juusola 1993). For the larger contrasts (0.6–1.0), the asymmetric nonlinearities became more obvious, whereupon the hyperpolarizing responses to light decrements were typically larger and slower than the depolarizing ones to light increments. For low-contrast responses at low-intensity backgrounds, the SNR was very low, and individual responses were noisy and difficult to distinguish from each other, even after being averaged 20 times. In the slowly adapting cells (sustaining 5–15 mV steady-state depolarization), the capability for contrast coding resembled that of adapting cells (Figs. 3, A and B), but their responses to positive contrasts were occasionally smaller, con-
very low coherence values. Resting potential during prolonged illumination—and after this, always had their initial behavior, also responded like this—i.e., hyperadapted close to dark (gray) for 14,000 photons/s. Note also that a majority of cells, regardless of instance, the highest coherence is for 1,400 photons/s and the second highest.

Note that the trace order does not follow the order of increasing intensity; for background intensity (14, 44, 140, 1400, 1,400, 4,400, and 14,000 photons/s). Among different cell categories. The poor coherence at the lowest background (red in A) unreliable.

Unexpectedly, most cells (14/23; ~61%), including many categorized originally as adapting or nonadapting, in fact, behaved quite similarly to the hyperadapting cells during prolonged (~90 s) illumination (Fig. 3C). The hyperadapting photoreceptors during the first second of their response to constant light (Fig. 1), all of these cells repolarized during the prolonged illumination with brightening background (~25 mV, 0 mV, +8 mV red, and +10 mV. Hyperpolarization was induced by current injection and depolarization by constant illumination (+8 mV; red) and current injection (+10 mV; black). C coherence functions for the data in B show high linearity and signal-to-noise ratio (SNR) in all voltages (~25, 0, and +10 mV; all black). Depolarization by light (+8 mV; red) decreases the coherence at lower frequencies by adding photon shot noise.

Fig. 5. Functional properties of the light-insensitive membrane in vivo are similar in all cockroach photoreceptors. A: a typical current-clamp recording (resting potential ~62 mV) shows slow charging of nearly passive membrane when hyperpolarized with current pulses (protocol below) and outward rectification when depolarized. B: examples of impedance functions for a typical photoreceptor illustrate this same behavior. In relation to the dark resting potential (~61 mV), the average membrane potentials from top to bottom are ~25 mV, 0 mV, +8 mV (red), and +10 mV. Hyperpolarization was induced by current injection and depolarization by constant illumination (+8 mV; red) and current injection (+10 mV; black). C coherence functions for the data in B show high linearity and signal-to-noise ratio (SNR) in all voltages (~25, 0, and +10 mV; all black). Depolarization by light (+8 mV; red) decreases the coherence at lower frequencies by adding photon shot noise.

Fig. 4. Linear filtering properties of LA cockroach photoreceptors investigated with white noise (WN)-modulated light stimulation also show differences among different cell categories. A: coherence functions show increase with brightening background (100, 320, 1,000, 10,000, 32,000, and 100,000 photons/s) in this adapting cell, as it did in all cells that maintained their steady-state depolarization during the whole illumination period at different levels of light adaptation. Here, the order of the traces follows brightening (red: the lowest intensity). Note that cells behaving like this were only a minority of hyperadapting cells always had very low values, irrespective of the adapting and slowly adapting photoreceptors.

A: Coherence functions show increase with brightening background (100, 320, 1,000, 10,000, 32,000, and 100,000 photons/s) in this adapting cell, as it did in all cells that maintained their steady-state depolarization during the whole illumination period at different levels of light adaptation. Here, the order of the traces follows brightening (red: the lowest intensity). Note that cells behaving like this were only a minority of hyperadapting cells always had very low values, irrespective of the adapting and slowly adapting photoreceptors. B: linear frequency-response function (gain and phase) for the example cell in A remains nearly constant with brightening (red: the lowest intensity). C: linear-impulse responses [1st-order Wiener kernels] were calculated for the data in B and are almost identical. The poor coherence at the lowest background (red in A) makes the estimates in B and C (red) unreliable. D: like this example, coherence functions of hyperadapting cells always had very low values, irrespective of the adapting background intensity (14, 44, 140, 440, 1,400, 4,400, and 14,000 photons/s). Note that the trace order does not follow the order of increasing intensity; for instance, the highest coherence is for 1,400 photons/s and the second highest (gray) for 14,000 photons/s. Note also that a majority of cells, regardless of their initial behavior, also responded like this—i.e., hyperadapted close to dark resting potential during prolonged illumination—and after this, always had very low coherence values.
beyond background intensities of frequency response functions between the WN-modulated light dynamics of contrast coding, we computed the coherence and quantify the response speed and reliability in terms of linear step-like contrasts does not increase in cockroach photoreceptors their encoding abilities.

Conclude that prolonged light exposure selectively abolishes after DA, generating large impulse and step responses, we conclude that prolonged light exposure selectively abolishes their encoding abilities.

Contrast-coding experiments showed that the coding of step-like contrasts does not increase in cockroach photoreceptors beyond background intensities of $\sim 1,000-10,000$ photons/s.

C coding of WN-modulated contrast in frequency domain. To quantify the response speed and reliability in terms of linear dynamics of contrast coding, we computed the coherence and frequency response functions between the WN-modulated light stimulus and the voltage response at different light backgrounds (Fig. 4). Because WN stimulation contains fast light changes around the mean, it has a tendency to linearize the output of insect photoreceptors (Faivre and Juusola 2008; Juusola and Hardie 2001; Juusola et al. 1994; Niven et al. 2004; Spekreijse and Oostings 1970). Here, the stimulus was changing fast enough to keep most responses within the linear range while still being sufficiently variable to induce occasional larger responses ($\sim 10-15$ mV). Similar to the results in the contrast-step experiments, we found that the contrast gain improved only marginally, if at all, with brightening intensity. Furthermore, the speed of responses was also practically invariant; the 3-dB cutoff frequency and the phase of the responses remained unchanged across the tested light backgrounds.

Like in contrast-step experiments above, a minority of the cells studied (six of 20; 30%) typically maintained $10-15$ mV steady-state depolarization during prolonged constant light exposure ($>1,000$ photons/s). Compared with the majority of cells, these photoreceptors had also markedly higher values of coherence ($>0.5$) at low stimulus frequencies (0.5–30 Hz)—in the best case, 0.8–0.9 at the brightest background (Fig. 4A). Thus apart from the dimmest photon shot noise-dominated backgrounds (<1,000 photons/s), their frequency-response estimates (Fig. 4B) were sufficiently reliable for comparative analysis. Their contrast gain (mV/unit contrast) generally increased only slightly, if at all, at low frequencies as a function of light intensity (Fig. 4B), and the variation of the 3-dB corner frequency of the cell (19–24 Hz) was small and failed to rise systematically with a brightening background. This suggested that the frequency response withstood LA largely unchanged. Because of lack of a systematic rise with brightness, the small variance in corner frequency is likely to be a result of the variance inherent in the computed estimate, due to the limited amount of data (Bendat and Piersol 1971), and hence, calculating the average corner frequency as a function of intensity would be misleading. The conclusion—that there were no systematic differences in temporal properties of the voltage responses among different levels of LA—was supported further by the astonishingly constant-phase functions (Fig. 4B), which are normally very sensitive to adaptive changes (Juusola et al. 1994). Finally, this constancy was quantified in time domain by calculating the linear-impulse responses from the frequency-response functions (Fig. 4C). When comparing different cells at the brightest backgrounds, where the coherence had the highest values, and the frequency responses were most reliable, the corner frequency varied between 15 and 25 Hz.

The majority of studied cells (14/20; 70%) was again, like in contrast-step experiments, found to behave like the hyper-adapting photoreceptors. Irrespective of the background brightness, they repolarized within 0–5 mV of the dark resting potential during long-lasting constant stimulation. Their coherence values were invariably very low (<0.4) in all tested light backgrounds, covering 3–5 log units and starting at about 10 photons/s (Fig. 4D). This implies that their responses either had a low SNR or were highly nonlinear (Bendat and Piersol 1971). It is reasonable to assume here that their low coherence mainly resulted from the low SNR, because when similarly LA, most photoreceptors (61%) failed to generate proper graded responses even to contrast steps (Fig. 3C). If anything, these photoreceptors produced only quantum bumps to positive contrasts. Because of the low coherence, the frequency-response
estimates were highly unreliable (Bendat and Piersol 1971) and were not evaluated further.

Experiments with WN stimulation again showed that the coding properties of photoreceptors do not change beyond ~1,000 photons/s and that with WN stimulation, the coding reliability at all light levels is relatively poor.

Membrane filtering. To find out how the properties of the nontransductive membrane are related to signal coding, we injected currents into the photoreceptors through the recording electrode using the switched-clamp technique. The input resistances of DA cells, when hyperpolarized (with a ~0.3-nA minus ~0.5-nA pulse) below the resting potential, were relatively large (60–180 MΩ) and increased with the level of hyperpolarization (Fig. 5A). The membrane charged very slowly; the time constants were between 20 ms and 50 ms in response to the negative current pulses. Conversely, depolarizing pulses revealed a strong rectification, which is probably caused by the activation of voltage-gated K⁺-channels, as in other studied insect photoreceptors (Hardie 1991; Laughlin and Weckström 1993; Niven et al. 2003; Vähäsöyrinki et al. 2006; Weckström et al. 1991; Weckström and Laughlin 1995).

The membrane impedance of the frequency response between the current input and voltage output (Fig. 5B) was determined with WN-modulated current injections (Juuosola and Weckström 1993; Weckström et al. 1992). The impedance behaved as expected from the current pulse experiments (Fig. 5A). When the membrane was hyperpolarized 20–30 mV below the dark resting potential, the impedance function (maximum 70–120 MΩ) followed the shape of a single-pole resistor–capacitor (RC) filter, with a corner frequency of 9 ± 1 Hz (mean ± SD; n = 19; Fig. 5B). At the dark resting potential, the maximum impedance was 40–60 MΩ and the corner frequency 20 ± 2 Hz (n = 22). When the membrane was depolarized, either by current injection or by constant illumination, the impedance at low frequencies dropped to ~10 MΩ, and the average corner frequency rose to 48 ± 7 Hz (n = 11). Again, these changes probably resulted from gradual activation of voltage-gated K⁺-channels. The corner frequency of the LA membrane (Fig. 5B) was about two times larger than that of the phototransduction output, i.e., light-induced voltage response (Fig. 4B). This suggested that the membrane does not limit the speed of the voltage responses in LA. No systematic association between the adapting properties (category) of the photoreceptor and the impedance of its membrane was found. Instead, all of the photoreceptor membranes behaved similarly within the above-given variation in corner frequency. The coherence functions indicated high linearity in all of the impedance recordings (Fig. 5C). When membrane was depolarized by constant light stimulation, the small drop in coherence values at low frequencies was most likely caused by photon shot noise.

With WN-modulated current and computation of the impedance functions, we could show that the DA membrane is very slow but does not limit the light-induced voltage responses under LA conditions.
SNR. Our previously published results (Heimonen et al. 2006) indicated that after a prolonged LA, the SNR(f) would be consistently very low (<0.1 in the majority of cells and maximally, ~0.3 in some cells at low frequencies). However, less was known about how SNR actually changes over time immediately following light stimulation. To address this question, DA cells were stimulated repeatedly by a 10-s-long WN-modulated contrast sequence. As seen in the response sequence (Fig. 6A), its modulation amplitude (i.e., the signal) was largest in the beginning and then diminished gradually. Further analysis in frequency domain, using only cells with the highest SNRs (Fig. 6B; n = 5), showed that the SNR(f) peaked for a few seconds after the stimulus onset, thereby implying that apart from the initial transient, the best performance of cockroach photoreceptors is in the early phases of LA. During the first 5 s of light stimulation, the SNR(f) had maximal values (>1) up to ~5 Hz, after which, it fell below 0.1 at 10–11 Hz. Additional analysis in the time domain (Fig. 6C; n = 13) quantified that the SNR(f) peaked on average for the first 5 s after stimulus onset and thereafter, rapidly decreased to the low levels reported previously (Heimonen et al. 2006) as the cells adapted to the mean light intensity.

These experiments showed the rapid decline of the SNR in photoreceptors when stimulated with WN stimulation, consistent with the results of experiments with contrast pulses.

Responses to naturalistic stimuli. Finally, to estimate the signaling performance of cockroach photoreceptors for more realistic stimuli, we recorded in vivo voltage responses of 12 photoreceptors to NIS. Fig. 7A shows representative responses of a slowly adapting photoreceptor, both after prolonged (~90 s) dark and LA. After DA, the NIS onset evoked a prominent depolarization, which decreased slowly over time, while being strongly modulated by the larger contrasts in the stimulus (NIS). After LA, the same cell produced a smaller response to the same NIS but still clearly followed the stimulus waveform and encoded the larger contrasts in it. In general, we found that faster-adapting cells reached this LA-encoding state more rapidly and hyperadapting cells immediately after the transient response or within the first second of stimulation. Thus all photoreceptors produced prominent voltage responses to NIS. Even the LA-hyperadapting cells generated up to 10 mV responses to the largest contrasts (>1) in it.

The coherence functions and the SNR(f)s of responses to NIS (Fig. 7B) were determined from nine photoreceptors both in DA and LA. In all of these cells, both functions were similar to the shown examples, although the hyperadapting photoreceptors had somewhat smaller values. Even though the coherence had higher values in LA, the SNR(f) was higher after DA. This was also seen during the first 5 s of the responses (Fig. 7A), where the modulation caused by the same stimulus sequence was notably larger after DA than in the LA state. This corresponds well with the WN results in Fig. 6. However, there were also prominent differences. The SNR(f) of responses to WN stimulation was always low—one to two at largest in low frequencies (<5 Hz; Fig. 6B)—whereas the SNR(f) of responses to NIS, presented here on a logarithmic scale (Fig. 7B), had ~100-fold higher low-frequency content (<5 Hz) and in many cases, contained more signal than noise (SNR > 1) up to ~10 Hz.

The rate of information transfer was estimated at five to seven different average light intensities in seven different cockroach photoreceptors (Fig. 7C) using the triple extrapolation method (Juusola and de Polavieja 2003). In brighter stimulation (>1,000 photons/s), hyperadapting cells had markedly lower information transfer rates than the more slowly adapting cells. One of the photoreceptors reached a relatively high rate of over 100 bits/s, already at an astonishingly low level of light intensity (~200 photons/s). The signaling performance of all recorded cells saturated in intensities between 100 and 10,000 photons/s.

To finalize the results, we compared the photoreceptor performance estimates obtained under different stimulation conditions and with different analyzing methods. In addition to information transfer rates during NIS stimulation, Fig. 7C shows estimates of Shannon (1948) information [\( C = \int \log_2(1 + SNR) \, df \)], achievable with the WN used (“WN limit”, calculated for the highest mean SNR(f) in Fig. 6B, and “linear limit” for the experiments with naturalistic stimuli, calculated for the higher SNR(f) in Fig. 7B). Therefore, WN limit approximates the achievable information capacity during WN stimulation, whereas the linear limit does the same when linear analysis is applied to the results of the NIS experiments.

These sets of experiments showed that whatever stimulation method is used, the contrast coding in cockroach photoreceptors seems to saturate near intensities of ~1000 photons/s.

DISCUSSION

With the use of various experimental and analytical approaches, we quantified functional properties of cockroach photoreceptors and showed how their signaling is selectively adapted for encoding light changes in dim naturalistic conditions. In the following, we consider the physiological adaptations of cockroach photoreceptors in specializing for the scotopic conditions.

Phototransduction gain. The gain of cockroach phototransduction is high, leading to production of large (~5–10 mV) quantum bumps (Fig. 1). Large bumps may be sufficient to drive the photoreceptor synapses, transmitting signals to deeper layers of the nervous system. In contrast, the quantum bumps are often small (~1 mV) in the few studied insect photoreceptors, including most diurnal flies (Dubs et al. 1981; Hardie 1979; Laughlin 1981; Wu and Pak 1975). Large quantum bumps have been found in rods of some vertebrate species (Baylor et al. 1979); in photoreceptors of nocturnal or crepuscular invertebrates, such as horseshoe crab Limulus (Dodge et al. 1968; Fuortes and Yeandle 1964), bee Megalopta (Frederiksen et al. 2008; Warrant 2008), and crane-fly Tipula (Laughlin 1996); and in locusts (Lillywhite and Laughlin 1979) that are active also in daylight. Hence, a large gain of phototransduction in dim conditions is obviously a necessary property for all animals active in such environments.

At bright light intensities, high phototransduction gain has the unwanted property of creating more voltage noise and thereby decreasing the SNR. In cockroach photoreceptors, the coherence and the SNR were poor at all light levels with WN stimulation (Figs. 4 and 6) (Heimonen et al. 2006), especially after long-lasting LA. The time course, during which the photoreceptor gain and SNR are decreased markedly when illuminated, is <10 s (Fig. 6). The contrast gain both in step and frequency responses was essentially unchanged above intensities of 1,000 photons/s (Figs. 3B and 4B), indicating that
phototransduction is saturated in some manner. A comparison of well-studied diurnal or crepuscular insects, such as *Calliphora* (Juusola et al. 1994) and *Drosophila* (Juusola and Hardie 2001; Niven et al. 2003), with a nocturnal bee *Melana* (Frederiksen et al. 2008) reveals a striking contrast. In all of these species, the bumps become smaller and faster, the gain diminishes, and the signaling bandwidth increases, while the SNR rises with increasing light up to daylight intensities. In the cockroach, the absence of these processes above 1,000 photons/s means that the photoreceptors adapt to bright light levels ineffectively. For the cockroach, the high gain at or near the quantum bump level seems to be paramount.

**Phototransduction speed and tuned filtering.** In dim conditions, visual signals are integrated over time, and accordingly, the photoreceptor response dynamics are slow (van Hateren 1992; Warrant 1999). This is also the case with the cockroach. The speed of voltage responses (Fig. 4C) and the charging properties of the DA photoreceptor membrane (Fig. 5B) are tuned similarly, both having ~20 Hz corner frequencies. This translates to an integration time constant of ~8 ms ($\tau = 1/2\pi f$, where $f = 3$ dB corner frequency) if a single-pole RC filter is assumed. Thus the temporal dynamics of phototransduction and the nontransductive membrane are matched approximately.

The slowness of light responses, as such, is not exceptional, since photoreceptors of other insect species are about as slow when dark adapted (Faire and Juusola 2008; Frederiksen et al. 2008; Howard et al. 1984; Juusola and Hardie 2001; Juusola et al. 1994; Laughlin and Weckström 1993; Niven et al. 2003). Surprising in the cockroach is that the speed of the voltage responses in bright light equals that in dim conditions. The usual finding of responses accelerating with increasing brightness was not observed. The response speed might increase slightly in some photoreceptors from a single-photon level up to ~1,000 photons/s but not beyond that. The constancy in response speed is best seen in the phase functions (Fig. 4B), which are normally the most sensitive indicators of adaptive changes. Concomitantly, also, the steady-state depolarization in constant light saturates at the same light level (Fig. 3B). Hence, cockroach photoreceptors are able to transduce the maximum of only ~1,000 photons/s, with only a minority of cells slightly exceeding that number. On the basis of the anatomy of cockroach retina, the number of microvilli is certainly much larger than 1,000 (Butler 1973b; Ferrell and Reitcheck 1993). The reason for the saturation of information rate at dim-light level has, therefore, to be sought from the bump production dynamics. Again, the well-studied phototransduction processes of the blowfly *Calliphora* (e.g., Howard et al. 1987; Juusola et al. 1994) and fruitfly *Drosophila* (e.g., Hardie and Raghu 2001; Juusola and Hardie 2001; Niven et al. 2003) contrast with this, showing clear changes in transduction speed and gain, at least up to intensities of ~10^5 photons/s. Accordingly, adaptive regulation of the gain and speed of cockroach phototransduction is limited to very low intensities. This conclusion is also supported by a recent study on fly photoreceptors, which provided strong evidence that bumps are likely to be generated in individual microvilli (transduction or sampling units), after which, these remain refractory for a variable period—unable to produce another bump even when hit by new photons (Song et al. 2012). According to those results, the ratio between activated and available microvilli and the timing and size of the bumps that they produce may be crucial in determining the photoreceptor’s information transfer rate and the intensity where it saturates. Accordingly, if microvilli in a cockroach photoreceptor generate large bumps and have long refractory periods, then even a relatively dim-light background would gradually render most of the microvilli refractory, saturating the photoreceptor output. This could explain why the signaling performance of cockroach photoreceptors to WN stimulation is so poor and improves only marginally with brightening.

However, when using NIS stimulation (van Hateren 1997), containing much higher contrasts and nearly 1/f frequency content, the photoreceptor performance becomes much better (Fig. 7, A and B), reaching a SNR of >100 and coherence of ~1 at low frequencies. Moreover, in contrast to the mostly indecisive results of WN experiments, the photoreceptor performance improves with increasing brightness of NIS stimulation. Information transfer rates can reach >100 bits/s with NIS stimulation, whereas they are only <20 bits/s during WN stimulation. For a purely linear measure, such as the one obtained from the SNR(f) by using the Shannon (1948) formula for information capacity, the estimated information transfer rate is limited below ~45 bits/s. The high rates (>100 bits/s) were obtained only with NIS stimulation and a novel information rate estimator (Juusola and de Polavieja 2003; Takalo et al. 2011), which does not assume linearity of the system or Gaussian distribution of the signals. Hence, the signaling mechanisms in cockroach photoreceptors appear to be highly adapted to naturalistic light changes, which contain long, darker periods that are likely to foster recovery of microvilli from the refractory state. Since the novel information rate estimates were clearly larger than the linear estimates, this adaptation is most likely achieved by using nonlinearities when tuning the photoreceptor signaling to code the changes of light intensity in natural environments. One such tuning mechanism is obviously formed by adaptation dynamics, and clearly, one future challenge is to elucidate optimal stimulus statistics and dynamics for the cockroach photoreceptors.

**Variability of cockroach phototransduction.** To explain the large variability of light responses, a specific form of population coding has been suggested to take place when cockroach photoreceptors are pooled for joint signal transmission (Heimonen et al. 2006). Most of the variability seems to originate in the phototransduction process, as was shown by the striking similarity of the dynamics of the voltage and current responses to light (Fig. 2). In addition, the photoreceptor sensitivity varied much less at the level of quantum bumps than at the level of full-fledged macroscopic responses. This suggests that cell-to-cell variation in the expression level of the light-gated channels is probably not the reason. The variability in properties of the nontransductive membrane of cockroach photoreceptors (Fig. 5) was also negligible. These findings suggest that much of the variability in macroscopic responses is introduced during so-called “bump summation”, where graded receptor potentials are summed up from single-photon signals and in the adaptation processes therein, rather than in the single-photon responses themselves. This summation process is well analyzed only in the case of *Limulus* photoreceptors (Wong and Knight 1980; Wong et al. 1980, 1982).

In the bump summation process, the generated bumps, each presumably arising from a single microvillus (Hardie and Raghu 2001; Howard et al. 1987; Song et al. 2012), and the
calcium signals, arising from the calcium influx during phototransduction (Hardie 2001; Hardie and Raghu 2001; Minke and Parnas 2006), are integrated by the cell soma and its membrane. For changes in the speed of this summation to occur, a mechanism is needed to change communication of microvilli with each other. Different calcium-dependent processes are probable candidates for this. They are also likely to be the major source of variability in the cockroach photoreceptors. Adaptation processes that could produce the variability include: translocation of the light-gated channels (Bähner et al. 2002; Meyer et al. 2006), concomitant changes in pH caused by protons released from their buffers by an increased intracellular calcium concentration (Huang et al. 2010), other calcium ion (Ca\(^{2+}\))-dependent enzymatic processes in phototransduction (Hardie 2001), and regulation of nontransductive membrane conductances (Krause et al. 2008).

Light responses of photoreceptors in cockroach and trp mutants. When observing the rapid repolarization of the cockroach photoreceptor light responses during continuing stimulation, one cannot avoid the comparison of this behavior with that of transient receptor potential (trp) mutants of D. melanogaster (Cosens and Manning 1969; Minke et al. 1975) and the nss-mutant (no steady-state) of the blowfly Lucilia (Barash et al. 1988; Howard et al. 1984; Suss-Toby et al. 1991). Both of these lack TRP channels, and consequently, the LIC is caused only by TRP-like (TRPL) channel activation. In these mutants, the voltage response to light drops rapidly toward the dark resting potential when stimulated with moderate-to-high light intensities. This phenomenon has been explained with rapid depletion of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)), a crucial membrane component in phototransduction that is the substrate of PLC, which is activated, via G-protein, by light (Hardie and Postma 2008). Normally, rising intracellular Ca\(^{2+}\) inhibits PLC (Hardie et al. 2001). In both mutants, the rapid decline of the light responses is due to the lack of TRP channels and the fact that light-activated current is flowing through TRPL channels only, leading to reduced influx of Ca\(^{2+}\) and therefore, unattenuated PIP\(_2\) depletion (Hardie et al. 2001). This line of reasoning suggests that in the cockroach, the light-gated channels could be more like trp channels in D. melanogaster, but this remains to be investigated.

Conclusions. We have shown that in cockroach photoreceptors, 1) the temporal properties of both phototransduction and nontransductive membrane are slow, 2) the adaptive changes are mostly limited to intensities <1,000 photons/s, 3) the high variability of the voltage responses originates in the variability of the LIC, and 4) voltage responses are nonlinearly tuned to naturalistic stimulation and perform poorly with step or WN stimulation.

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DISCLOSURES
The authors declare no potential conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

REFERENCES


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