Signal coding in cockroach photoreceptors is tuned to dim environments


1University of Oulu, Department of Physics, Oulu, Finland, 2University of Sheffield, Department of Biomedical Science, Sheffield, UK, 3State Key Laboratory of Cognitive Neuroscience, Beijing Normal University, Beijing, China

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Address for correspondence:
Matti Weckström, University of Oulu, Department of Physics, Biophysics, FIN-90014 University of Oulu
Email: matti.weckstrom@oulu.fi
Abstract

In dim light, scarcity of photons typically leads to poor vision. Nonetheless, many animals show visually guided behaviour with dim environments. We investigated the signaling properties of photoreceptors of dark-active cockroach (*Periplaneta americana*), using intracellular and whole-cell patch-clamp recordings, to determine whether they show selective functional adaptations to dark. Expectedly, dark-adapted photoreceptors generated large and slow responses to single photons. However, when light-adapted, responses of both phototransduction and the non-transductive membrane to white-noise modulated stimuli remained slow, with corner-frequencies ~20 Hz. This promotes temporal integration of light inputs and maintains high sensitivity of vision. Adaptive changes in dynamics were limited to dim conditions. Characteristically, both step and frequency responses stayed effectively unchanged for intensities >1,000 photons/s/photoreceptor. Signal-to-noise ratio of the light responses was transiently higher at frequencies <5 Hz for ~5 s after light-onset, but deteriorated to a lower value upon longer stimulation. Naturalistic light stimuli, as opposed to white-noise, evoked markedly larger responses with higher signal-to-noise ratios at low frequencies. This allowed realistic estimates of information transfer rates, which saturated at ~100 bits/s at low light intensities. We found, therefore, selective adaptations beneficial for vision in dim environments in cockroach photoreceptors: large amplitude of single photon responses, constant high level of temporal integration of light inputs, saturation of response properties at low intensities, and only transiently efficient encoding of light contrasts. The results also suggest that the sources of the large functional variability between different photoreceptors reside mostly in phototransduction processes, not in the properties of the non-transductive membrane.

Keywords: Vision, systems analysis, adaptation, temporal resolution, photons
Introduction

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; Weckström and Laughlin 1995; Warrant 1999, 2004). 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 (e.g. 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 non-transductive 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 (Lillywhite and Laughlin 1979; Laughlin and Lillywhite 1982). Dark noise (spontaneous bumps without light), which may be significant in vertebrate vision (Aho et al. 1988), is of minor importance in invertebrates (Lillywhite 1977; Laughlin and Lillywhite 1982; Heimonen and Weckström, unpublished observations), and likely to be specifically suppressed by a molecular mechanism within the phototransduction cascade (Katz and Minke 2012).

Vision generally improves with increasing brightness. Light adaptation makes phototransduction faster, quantum bumps smaller and their latencies shorter (Howard et al. 1987; Juusola et al. 1994). Concomitantly, transduction gain (response amplitude per unit of stimulus intensity) decreases, though contrast gain (response amplitude per unit of stimulus contrast) increases. Such signaling dynamics have been described in detail in several insects (e.g. Juusola et al. 1994; Juusola and Hardie 2001; Niven et al. 2003; Faivre and Juusola 2008). With light adaptation, 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 (e.g. Laughlin 1990; Warrant and McIntyre 1993; Warrant 1999, 2006, 2008). 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 (e.g. Laughlin 1990; Warrant 1999, 2004). In contrast, apposition compound eyes, like those in the cockroach (Butler 1971, 1973b), may be thought to be inadequate for night vision. However, cockroaches are active mainly under dim conditions (Roberts 1965; Guthrie and Tindall 1968; Lipton and Sutherland 1970). They tend to avoid light when given a choice (Guthrie and Tindall 1968; Kelly and Mote 1990; Halloy et al. 2007), directing their escape responses toward dark places (Riemay 1984; Ye et al. 2003). In addition, there are also other nocturnal species, which have apposition eyes (review: Warrant, 2008). Contrary to compound eyes in general, cockroach eyes have a highly irregular structure (Butler 1973a, 1973b; Ribi 1977; Ernst and Fuller 1987). 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 using both
white-noise 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 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 about 30 minutes. 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 (Weckström et al. 1993; Heimonen et al. 2006). 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, the both retinæ were cut into pieces 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 Corp., Lakewood, NJ USA) and 0.2 mg/ml pankreatin (Sigma-Aldrich) for 10 min incubation before the actual dissociation. The dissociation of ommatidia was done by triturating the enzymatically treated pieces with a series of 3 to 4 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 in 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 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, USA) 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 centre. In this configuration, the LED spatially subtended about 2° at the photoreceptor level. The current stimulus waveforms were also computer-generated 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, USA) based custom-written software (Co. M. Juusola), running on a computer equipped with a data acquisition card (National Instruments PCI-MIO-16E-4, USA). The monitored stimuli and the amplified responses were low-pass filtered at 250, 500 or 700 Hz (Kemo VBF/23, USA) and sampled at 0.5, 1.0 or 1.2 kHz.
**Setup for in vitro experiments**

The whole-cell patch-clamp recordings were performed in 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, 10 N-Tris-(hydroxymethyl)-methyl-2-aminooethanesulfonic acid (TES), 2 MgCl₂, 4 Mg-ATP, 0.4 Na-GTP and 1 NAD (pH adjusted to 7.15 with KOH). 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 NaOH). The liquid junction potential between the solutions (~ -4 mV) was considered negligible for these recordings. Signals were amplified with Axopatch 1-D amplifier (Molecular Systems, USA), filtered with an 8-pole Bessel filter, and recorded, sampled and analyzed with PClamp 9 software (Molecular Systems, USA). 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 microscope 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 per second (photons/s) (cf. Juusola et al. 1994; Heimonen et al. 2006). 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 either pulses with different intensities, contrasts and durations, or pseudorandomly modulated contrast sequences (filtered Gaussian white-noise, WN), both at different intensities of background light (a more complete account of these stimuli is reported in Juusola 1993; Juusola et al. 1994; Kouvalainen et al. 1994). Alternatively, the waveforms could be time series of naturalistic light intensity variation (naturalistic intensity series, NIS) selected from the van Hateren’s natural image database (van Hateren 1997). When light-adapted responses were recorded, the originally dark-adapted 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 (see e.g. Butler 1971; Snyder and Horridge 1972; Butler 1973b; Ferrell and Reitcheck 1993). Long periods of light stimulation, especially with bright intensities, were always followed by dark adaptation for several minutes. The length of dark adaptation 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 \(\Delta I\) divided by the mean light background \(I\). In the case of pseudorandom contrast modulation, the standard deviation \(SD\) 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 photo-insensitive 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 > 60 MΩ (tested with a hyperpolarizing -0.3 nA current pulse at the dark resting potential), and have clearly visible...
quantum bumps with amplitudes of several millivolts (cf. Fig. 1) at low light intensities. Typically the resting potential was \(-60\) 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 dark adaptation. In vitro whole-cell recordings had to fulfill the following criteria to be selected into this study: resting potential \(\leq -50\) 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, 1980b), as well as the signal-to-noise ratio 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 (1st order Wiener kernels) were obtained from the frequency responses via the inverse fast Fourier transform. Details of how to measure cell impedances in vivo, utilizing WN modulated current injections, are reported earlier (Weckström et al. 1992; Juusola and Weckström 1993). The coherence functions between NIS and photoreceptor voltage responses, and the SNR\((f)\) of these responses, were also recorded and analyzed as described earlier (Juusola et al. 1994; Kouvalainen et al. 1994; Heimonen et al. 2006). Here, ten 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 times 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 holding potential of -70 mV) of the amplifier in the same cells to justify direct comparison of their waveforms. SNR of responses (recorded in \(I = 0\) mode) to a repeated (10-15 times) 10-s-long WN modulated contrast sequence (\(c = 0.32\)) was estimated in two ways. 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) were detrended by using a 6th order polynomial fit. The SNR\((f)\) was first analyzed as previously reported (Kouvalainen et al. 1994). Then, in time-domain, SNR\((t)\) was estimated in the following way: (1) the detrended responses were divided into ten equal length time segments (ten 0.9 s bins), (2) the corresponding response segments were averaged to give an estimated signal, (3) the variance of this signal was divided by that of noise (the difference between the signal and an original detrended response) to obtain SNR\((t)\) of each segment. In the SNR recordings, the mean light intensity was always bright enough to produce saturated responses in dark-adapted 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: “hyper-adapting”, “adapting” and “non-adapting” typify two extremes and an intermediate behavior of an actually continuous distribution of response variability (cf. 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 dark-adapted photoreceptors. The light-induced current responses (Fig. 2) were examined in order to find out, if the source of the variability of the light voltage responses is in the light-induced current. Then we examined how light-adapted 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 further investigated by recording and analyzing responses to white-noise modulated contrast sequences at different adapting backgrounds (Fig. 4). The electrical properties of the light-insensitive photoreceptor membrane were quantified in order to examine their contribution to coding (Fig. 5). Finally, to find out the photoreceptor performance at various light levels, we measured the signal-to-noise ratio, both the $SNR(t)$ and the $SNR(f)$, of voltage responses after the lights onset (Fig. 6) and computed the rate of information transfer for naturalistic light intensity series (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 dark adapted photoreceptors

The dark-adapted voltage responses were recorded first, to show the variability as described before (Heimonen et al 2006), and secondly, to further study the light adaptation 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 approximately 100-fold sensitivity differences, as determined by their individual V-logI curves (Weckström et al. 1993; Heimonen et al. 2006). With longer light pulses, differences in adaptation emerged (Figs. 1A, 1C).

Whilst the early transient responses of different cells (Fig. 1C) had similar shapes, they repolarized differently during constant light. Cells that were earlier categorized as “non-adapting”, using 300 ms stimuli (Heimonen et al. 2006), responded actually with slow repolarization towards dark resting potential, when longer 10-s-light pulses were used (Fig. 1, right column). Hence, they are better termed “slowly-adapting”. The left column of Fig. 1 shows responses of the “hyper-adapting” 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, left column). Thus the “hyperadaptation” is more like reduced excitation efficiency than adaptation of bumps. Although here found to be a misnomer, the “hyper-adapting” 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 (Weckström et al. 1993; Heimonen et al. 2006), the differences in the absolute sensitivity were ~10-times less. With the same dim background, the average bump frequency of different cells varied between 2 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 towards the resting potential.

**Light induced currents and corresponding voltage responses**

To search for the sources of functional variability, we recorded light-induced currents (LIC) 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 (top and bottom traces in each column of Fig. 2), we found that their waveform dynamics were nearly equivalent in each of the cells. For example, the voltage response of a “hyper-adapting” cell closely followed the time course of its LIC (the leftmost traces). These results suggest that (i) the variability in voltage responses reflects the variability in the phototransduction process (LIC), and that (ii) the role of the non-transductive membrane properties in shaping the response waveforms is limited compared to 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 (9/23) sustained their steady-state depolarization markedly (5-15 mV) above the dark resting potential (Figs. 3A, 3B). 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 (8/9 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 signal-to-noise ratio 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. 3A, 3B), but their responses to positive contrasts were occasionally smaller, containing randomly overlapping quantum bumps (Fig. 3C, top set of traces). Naturally, this noisy nature reduced the amplitude of averaged responses.

Unexpectedly, most cells (14/23; ~61%) – including many originally categorized as “adapting” or “non-adapting” – in fact behaved quite similarly to the “hyper-adapting” cells during prolonged (≥90 s) illumination (Fig. 3C, bottom set of traces). Like the “hyper-adapting” photoreceptors
during the first second of their response to constant light (Fig. 1, left column), all these cells repolarized during the prolonged illumination within 0-5 mV of the dark resting potential. Consequently, these photoreceptors completely failed to encode negative contrasts (Fig. 3C, bottom set of traces), and did this irrespective of the background intensity. Additionally, only some of them could respond to positive contrasts ($c \leq 1$) with random and sparse bump-like responses (similar to Fig. 1B, left column), which were lost in averaging. As a result, the majority of cockroach photoreceptors (here 61% of tested cells) could not encode contrast steps below unity ($-1 \leq c \leq 1$), when light-adapted for long enough time. Because these cells could encode light changes after dark adaptation, 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 ~1000-1,0000 photons/s.

**Coding of white noise 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 (Spekreijse and Oostings 1970; Juusola et al. 1994; Juusola and Hardie 2001; Niven et al. 2004; Faivre and Juusola 2008). Here, the stimulus was changing fast enough to keep most responses within the linear range whilst still being sufficiently variable to induce occasional larger responses (~10-15 mV). Similarly 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 cut-off 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 (6/20; 30%) maintained typically 10-15 mV steady-state depolarization during prolonged constant light exposure (>1,000 photons/s). When compared to 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, upper panel), whilst the variation of the 3 dB corner-frequency of the cell (19-24 Hz) was small, and failed to rise systematically with brightening background. This suggested that the frequency response withstood light adaptation largely unchanged. Because of lack of 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 between different levels of light-adaptation, was further supported by the astonishingly constant phase functions (Fig. 4B, lower panel), which are normally very sensitive to adaptive changes (e.g. 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%) were 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 non-linear (Bendat and Piersol 1971). It is reasonable to assume here that their low coherence mainly resulted from the low SNR, because when similarly light-adapted, 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 further evaluated.

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 non-transductive 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 dark-adapted cells, when hyperpolarized (with a -0.3 to -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 20-50 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 (Weckström et al. 1991; Hardie 1991; Laughlin and Weckström 1993; Weckström and Laughlin 1995; Niven et al. 2003; Vähäsöyrinki et al. 2006). The membrane impedance, or the frequency response between the current input and voltage output (Fig. 5B), was determined with WN modulated current injections (Weckström et al. 1992; Juusola and Weckström 1993). 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 (max. 70-120 MΩ) followed the shape of a single-pole 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 about 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 light-adapted membrane (Fig. 5B; red) 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 light adaptation. No systematic association between the adapting properties (category) of the photoreceptor and the impedance of its membrane was found. Instead, all the photoreceptor membranes behaved similarly, within the above given variation in corner-frequency. The coherence functions indicated high linearity in all 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 dark-adapted membrane is very slow but does not limit the light-induced voltage responses under light-adapted conditions.
Signal-to-noise ratio

Our previously published results (Heimonen et al. 2006) indicated that after a prolonged light adaptation, the SNR(f) would be consistently very low; <0.1 in the majority of cells, and maximally \( \sim 0.3 \) 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, dark-adapted cells were repeatedly stimulated by a 10-s-long WN modulated contrast sequence. As seen in the response sequence (Fig. 6A; top trace), 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 light adaptation. During the first five seconds of light stimulation, the SNR(f) had maximal values (>1) up to \( \sim 5 \) 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(t) peaked on average for the first five seconds 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 of 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 twelve photoreceptors to naturalistic light intensity series (NIS). Fig. 7A shows representative responses of a “slowly-adapting” photoreceptor, both after prolonged (\( \geq 90 \) s) dark and light adaptation. After dark adaptation (black trace, DA), the NIS onset evoked a prominent depolarization, which slowly decreased over time, while being strongly modulated by the larger contrasts in the stimulus (black bottom trace; NIS). After light adaptation, the same cell produced a smaller response (red trace, LA) 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 light-adapted encoding state more rapidly, and “hyper-adapting” cells immediately after the transient response, or within the first second of stimulation. Thus, all photoreceptors produced prominent voltage responses to NIS. Even the light-adapted “hyper-adapting” 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 dark (DA, black) and light adaptation (LA, red). In all these cells, both functions were similar to the shown examples, although the “hyper-adapting” photoreceptors had somewhat smaller values. Even though the coherence had higher values in light adaptation, the SNR(f) was higher after dark adaptation. This was also seen during the first five seconds of the responses (Fig. 7A), where the modulation caused by the same stimulus sequence was notably larger after dark adaptation than in the light-adapted 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, being 1-2 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, lower panel), had about 100-fold higher low frequency content (<5 Hz), and in many cases contained more signal than noise (SNR >1) up to \( \sim 10 \) Hz.
The rate of information transfer was estimated at 5-7 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), “hyper-adapting” cells (grey labels and lines) had markedly lower information transfer rates than the more slowly adapting cells (black labels and lines). 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 (marked “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, while 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

Using 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 (Wu and Pak 1975; Hardie 1979; Dubs et al. 1981; Laughlin 1981). Large quantum bumps have been found in rods of some vertebrate species (Baylor et al. 1979); in photoreceptors of nocturnal or crepuscular invertebrates, like horseshoe crab Limulus (e.g. Fuortes and Yeandle 1964; Dodge et al. 1968); bee Megalopta (Frederiksen et al. 2008; Warrant 2008); crane-fly Tipula (Laughlin 1996); and in locusts (Lillywhite and Laughlin 1979) that are active also in daylight. Hence, 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, 6; Heimonen et al. 2006), especially after long lasting light adaptation. The time course, during which the photoreceptor gain and SNR are decreased markedly when illuminated, is less than 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, 4B), indicating that phototransduction is saturated in some manner. A comparison to well-studied diurnal or crepuscular insects, like Calliphora (Juusola et al. 1994) and Drosophila (Juusola and Hardie 2001; Niven et al. 2003), and a nocturnal bee Megalopta (Frederiksen et al. 2008), reveals a striking contrast. In all 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 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 (cf. 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 dark-adapted photoreceptor membrane (Fig. 5B) are tuned similarly, both having ~20 Hz corner-frequencies. This translates to an integration time constant of ~8 ms (τ = 1/2πf, where f = 3 dB corner-frequency), if a single-pole RC-filter is assumed. Thus, the temporal dynamics of phototransduction and the non-tranductive 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 (Howard et al. 1984; Laughlin and Weckström 1993; Juusola et al. 1994; Juusola and Hardie 2001; Niven et al. 2003; Faivre and Juusola 2008;
Frederiksen et al. 2008). 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 a little in some
photoreceptors from 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 1000 (Butler 1973b; Ferrell and Reitheck 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 ~10^6 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 they produce may be crucial
in determining 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 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 (Figs. 7A, 7B),
reaching 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,
while 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; see also 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 utilizing non-
linearities 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 non-transductive 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 (Howard et al. 1987; Hardie and Raghu 2001; 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 Ca^{2+}-dependent enzymatic processes in phototransduction (Hardie 2001), and regulation of non-transductive membrane conductances (e.g. Krause et al. 2008).

Light responses of photoreceptors in cockroach and trp-mutants

When observing the rapid repolarization of the of the cockroach photoreceptor light responses during continuing stimulation, one cannot avoid the comparison of this behavior to that of trp-mutants of Drosophila melanogaster (Cosens and Manning 1969, Minke et al. 1975) and the nss-mutant of the blowfly Lucilia (Howard et al. 1984, Barash et al. 1988, Suss et al. 1991). Both of these lack TRP (Transient Receptor Potential) channels, and, consequently, the LIC is caused only by TRPL (TRP-like) channel activation. In these mutants the voltage response to light drops rapidly towards the dark resting potential when stimulated with moderate-to-high light intensities. This phenomenon has been explained with rapid depletion of PIP_2 (phosphatidylinositol 4,5-bisphosphate), a crucial membrane component in phototransduction that is the substrate of PLC (phospholipase-C), 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 trpl-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 non-transductive 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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Author Contributions

K.H. made most of the in vivo recordings and analyzed them, made the figures and wrote the original draft manuscript; E.-V.I. and R.F. made the in vitro recordings and analyzed them; I.S. made some in vivo recordings and helped with their analysis; M.J. calculated the rates of information transfer; M.V. and M.W. introduced ideas and supervised the work; M.W. participated in drafting the manuscript. All authors participated in editing the final manuscript.
Figure captions:

**Figure 1.** Dark-adapted cockroach photoreceptors produce variable voltage responses to light steps in vivo. Cells with different types of responses can be categorized according their adaptation dynamics: “hyper-adapting” (left column), “adapting” (middle column), and “slowly or non-adapting” (right column). A. Typical saturated voltage responses to 300 ms light pulses in each category. B. Examples of single photon responses (quantum bumps) during constant illumination. Even during bright illumination, “hyper-adapting” responses often show bump-like events (left) while repolarizing close to the dark resting potential (box and arrow). “Adapting” and “slowly-adapting” cells (middle and right) generate clearly distinguishable single photon responses (here 3 and 17 bumps/s) only during dim illumination. C. Saturated responses to bright 10-s-long light pulses (timing shown between the response rows) exemplify the variability in adaptation time course and level of depolarization between different types of cells. The dotted lines below the responses indicate the dark resting potential (ca. -60 mV in each cell).

**Figure 2.** In vitro whole-cell patch-clamp recordings of dark-adapted photoreceptors show similar variability both in voltage responses (top row) and in 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 the responses. The transient early responses of the LICs are clipped because of their large amplitude.

**Figure 3.** Light-adapted photoreceptors of different categories produce different voltage responses to positive and negative contrast pulses in vivo (all recordings are 10-20 times averaged). A. In a typical “adapting” cell, responses to contrast stimuli (protocol at the bottom; -1 ≤ c ≤ 1) change both in amplitude and in shape as a function of the background intensity (given in right as photons/s). Changes are especially obvious between the low intensity backgrounds. In addition, the average “steady-state” depolarization (given in left; from the dark resting potential of -60 mV) increases with the intensity of the background. B. $V$-log$I$ curves for the data in A depict the changes in the maximum amplitude of the contrast response (solid lines connect the data points at each background) and in the background-induced “steady-state” potential (dotted lines). Note that both the “steady-state” depolarization and the contrast responses (whole $V$-log$I$ curves) saturate already ~1,000 photons/s. C. Examples of similar contrast step responses in different cell categories. Stimulus protocol was the same, and the background intensities and “steady-state” potentials are presented, as in A. Note that in some (not all) “slowly-adapting” cells the positive responses were very small, and in “hyper-adapting” cells all the responses nearly non-existent.

**Figure 4.** Linear filtering properties of light-adapted cockroach photoreceptors investigated with WN modulated light stimulation show also differences between 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, which 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 “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 “hyper-adapting” 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, responded also like this, i.e. “hyper-adapted” close to dark resting potential during prolonged illumination, and after this had always very low coherence values.

**Figure 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 of 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 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.

**Figure 6.** SNR of cockroach photoreceptors decreases markedly during prolonged light stimulation *in vitro*. **A.** A typical example voltage response (middle trace) to a bright WN modulated stimulus (bottom trace; $I_{\text{mean}} \approx 5000$ ph/s) after dark adaptation resembles the waveform of a response to a light step (cf. Figs. 1, 2). The transient response (first second) is omitted from the SNR analysis. The subsequent overall waveform (1 – 10 s) is fitted and the result is subtracted from the original response to leave 9 s of WN modulation induced response with zero mean (top trace). **B.** Mean $SNR(f)$ with SD bars ($n = 5$) is shown for three time periods: 1 - 5.5 s (black); 5.5 – 10 s (grey) and 1 – 10 s (red; SD bars omitted here for clarity). Only photoreceptors with higher SNR ($\geq 1$) at low frequencies were chosen for this analysis. **C.** The mean $SNR(t)$ (presented with SD bars; $n = 13$), during different time intervals (ten 0.9 s bins) of the WN stimulus, shows clear and continuous drop after the first 5 s.

**Figure 7.** Stimulation with naturalistic light intensity series (NIS) evokes noticeably higher SNR and information transfer rate than WN stimulation. **A.** Voltage responses from dark- (DA, black) and light-adapted (LA, red) photoreceptors to NIS ($I_{\text{mean}} \approx 5,000$ ph/s) carried much larger voltage variations than corresponding WN induced responses (cf. Fig. 6A). The power spectrum of the stimulus (on right) followed roughly the $1/f$-shape of intensity variation found in natural scenes. **B.** Coherence functions and $SNR(f)$s (averages for 10 recordings like in A, color as in A) had markedly higher values, especially at low frequencies, than corresponding functions for WN stimulation (cf. Figs. 4A, 4B, 6B and 6C). **C.** Information transfer rate (mean with SD bars) as a function of average light intensity in all seven different photoreceptors is clearly higher than ever in WN experiments. Cell specific intensity values were obtained with bump calibration for each photoreceptor, separately. Grey symbols and lines represent three rapidly and strongly adapting cells, of which one was “hyper-adapting” (the lower-most trace). Black symbols and lines represent four more slowly adapting cells, similar to the example cell in A. “WN limit” for the information transfer rate (13 bits/s, Shannon information capacity) is calculated for the highest mean $SNR(f)$ in Fig. 6B (1-5.5s, black) and the linear limit (45 bits/s) for the higher $SNR(f)$ in B (DA, black in lower panel).


"hyper-adapting" | "adapting" | "slowly/non-adapting"

A

B

C

20 mV | 200 ms

2 mV | 200 ms

20 mV | 2 s
A. "Adapting"
- 13 mV, 320 000 ph/s
- 12 mV, 32 000 ph/s
- 14 mV, 3200 ph/s
- 11 mV, 1000 ph/s
- 10 mV, 100 ms
- 5 mV, 320 ph/s
- 4 mV, 100 ph/s

B. Graph showing membrane voltage (mV) vs. light intensity (ph/s).

C. "Slowly-adapting"
- 15 mV, 2800 ph/s
- 10 mV, 100 ms
- 0 mV, 3000 ph/s

"Hyper-adapting"