BIOPHYSICS OF NIGHT VISION

Cockroach (Periplaneta americana) Photoreceptors as a Model System

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dark night poem

yey say that
nothing is wasted:
either that
or
it all is.

- Charles Bukowski -
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**Abstract**

Photoreceptors convert the energy of light into an electric signal to be processed by the visual system. Photoreceptors of nocturnal insects are adapted for night vision by sacrificing spatial and temporal resolution for improved sensitivity. While the sensitivity-increasing optical adaptations and the temporal properties of light responses have been studied earlier, the intermediate biophysical mechanisms responsible for shaping the captured light into voltage responses were previously not known in detail in any nocturnal species.

Using electrophysiological tools and computer simulations the photoreceptors of the nocturnal cockroach (*Periplaneta americana*) were studied by characterising 1) the electrical properties responsible for shaping the light responses, 2) the properties of light responses at different stages of light and dark adaptation and 3) properties of low-intensity light stimuli and how they are processed by the photoreceptors.

The high input resistance and whole-cell capacitance were typical for a nocturnal insect, but the two voltage-dependent potassium conductances were closer to those found in diurnal species. The dominant sustained conductance typically associated with day-light vision activated during simulated light responses whereas the lesser transient conductance previously linked to low-light vision did not. Light responses were persistently slow regardless of the adapting light level and saturated at low intensities, indicating a strong adaptation to vision in dim light. Simulations showed that at such low light levels the physical noise caused by random photons determines the information rate and the biological noise, caused by random latency and amplitude of single photon responses, has only a minor effect. At higher intensities the latency variability degraded the information rates but the amplitude variability did not. Thus, photoreceptors of nocturnal animals can sacrifice phototransduction precision in their natural illumination without compromising their coding performance.

*Keywords*: vision, photoreceptor, ion channel, information
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LIST OF ABBREVIATIONS

1/f signal whose power is inversely proportional to frequency
a_x activation parameter for voltage-dependent potassium conductance x
a_{ss x} steady-state activation parameter for voltage-dependent potassium conductance x
C capacitance (in Siemens) or Shannon information rate (in bits/s)
CLC-2 a chloride channel belonging to the CLC voltage-gated chloride channels family
f_c cut-off frequency
g conductance
G (prefix) giga, $10^9$; or alternatively maximum conductance, e.g. $G_{KDR}$
g_{LIGHT} light-dependent conductance
GWN Gaussian white noise
i_{KA} inactivation parameter for the transient voltage-dependent potassium conductance
IR the inward-rectifying conductance in cockroach photoreceptors
i_{ss KA} steady-state inactivation parameter for the transient voltage-dependent potassium conductance
KA the transient voltage-dependent potassium conductance in cockroach photoreceptors
KDR the sustained voltage-dependent potassium conductance in cockroach photoreceptors
K_v voltage-dependent potassium conductance
LED light-emitting diode
LIC light-induced current
LMC large monopolar cell
lvf long visual fibre
m (prefix) milli, $10^{-3}$
M (prefix) mega, $10^6$; or alternatively mol/l in concentration
n (prefix) nano, $10^{-9}$; or alternatively the number of trials/cells in a dataset
NIS natural intensity series light stimulus
P power spectrum ($P_S$: signal power; $P_N$: noise power; $P_{xy}$ cross-power spectrum)
PIP2  phosphatidylinositol 4,5-bisphosphate
PLC  phospholipase-C
R  resistance
R_{in}  input resistance
ROC  Receiver-operating characteristic
SNR  signal-to-noise-ratio
svf  short visual fibre
t  time
TRP,  transient receptor potential and transient receptor potential –like
TRPL  channel
UV  ultra violet
V  voltage, membrane potential
V_{50}  half-activation potential for the steady-state activation/inactivation function
\gamma^2(f)  coherence function
\lambda  mean value of Poisson random process
\tau  time constant
LIST OF ORIGINAL ARTICLES


Contribution:

In paper (I) I made a mathematical model of a cockroach photoreceptor based on whole-cell recordings done by Stephan and Yani Krause, Esa-Ville Immonen and Roman Frolov and I was responsible for writing the paper. In paper (II) I recorded light responses from cockroach photoreceptors at different states of light adaptation, analysed the responses and participated in writing. In paper (III) I modelled the propagation of information by shot noise input through a noisy photoreceptor, performed intracellular recordings and wrote the first draft of the paper; Jouni Takalo calculated the information rate estimates. Complete author contributions are listed in the papers.

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1 Introduction

For most animals, light is the most versatile source of information about their surroundings. Various forms of visual systems have evolved to exploit the different cues offered by the intensity, wavelength and polarization of light. All of these features are used by insects, which have successfully invaded most of the terrestrial niches found on Earth. This thesis deals with vision of the cockroach *Periplaneta americana*, a semi-urban nocturnal pest insect that has spread all over the world.

The darkness of night can offer safety from predators and reduce competition for food and other resources between different species. On the other hand, being nocturnal demands a great deal from the visual system, as information must be collected from light that can be 100 million times dimmer than what is available during daytime in the same area. Regardless of this challenge, many animals have adapted a nocturnal lifestyle and their visual systems are specialized in sensing - as well as making sense of - low-intensity light signals.

In addition to its wave-like nature, light is particulate, that is, consisting of photons and the intensity of objects we see is proportional to the mean number of photons they reflect (or emit, if looking at light sources). In bright light a steadily illuminated object appears to be reflecting a steady intensity, but with low enough illumination so few photons are reflected that they are sampled by the eye as separate events. The emission and absorption of photons are random quantum processes and hence constitute an unreliable signal called shot noise. While we limit most of our activities to conditions where light is abundant, many animals are active under intensities where shot noise reigns and visual systems are forced to function under the physical limit set by shot noise photons. In dim light, such as natural illumination at night, shot noise makes seeing difficult due to the scarcity and randomness of photons. Nevertheless, shot noise is often the very signal that photoreceptors of nocturnal animals must cope with in order to see anything at all.

Many animals actively use their vision on the darkest night. What kinds of structural and functional features are required from their visual systems? Obviously, collecting more light by the eyes helps. Compared to their diurnal relatives, nocturnal animals have eyes with more sensitive optics that are able to collect light with a larger pupil area, and more sensitive photoreceptors which can capture the photons collected by the optics more efficiently. However, these
adaptations alone are often not enough, and efficient signal processing is necessary for seeing in the dark. To make things even harder, the same visual system has to function in bright light as well.

To function during both day and night, the visual system must be able to extract information from a visual input covering a vast range of light intensities. For this task vertebrates have two types of photoreceptors: rods and cones, which are responsible for day and night vision, respectively, and have different biophysical properties (Rodieck 1998). The larger size and higher phototransduction gain of rods allows efficient photon capture and generation of large single photon responses, both of which are necessary for night vision. The kinetics of rod light responses are slow, enabling temporal integration of absorbed photons over long time windows. Rods also contain specific voltage-gated ion conductances which shape the responses to low-light stimulation (Beech & Barnes 1989). Altogether the biophysical properties of rods ensure that the signals generated by low-intensity stimuli are delivered to the second order cells reliably.

While the cone photoreceptors used for daylight vision are far less sensitive than rods, they have much better temporal and spatial acuity (Rodieck 1998). Cones also come in more than one variety of spectral sensitivity (three for most of us humans), allowing colour vision. But whereas vertebrates have different photoreceptor types dedicated for night and day vision, most insects have to cope with just one type, possibly with some modifications (Anderson & Hardie 1996, Anderson & Laughlin 2000, Cuttle et al. 1995, Hardie 1979). The obvious question is what kind of biophysical properties are required from a single photoreceptor type that takes responsibility for vision at all hours of the day? Whereas plenty of research on the subject has been done on e.g. flies (Juusola et al. 1994, Juusola & Hardie 2001, Juusola & de Polavieja 2003, Juusola et al. 2003, Niven et al. 2007, Song et al. 2012, Weckström et al. 1991), little is known about nocturnal insects.

The purpose of light- and dark-adaptation is to improve the photoreceptor coding performance under the prevailing illumination. In diurnal animals that are active in bright light, light-adaptation prevents saturation of the photoresponses and may improve the signalling performance by e.g. accelerating the time course of responses. Currently the effects of light and dark-adaptation in photoreceptors that have evolved to function mainly in low-light conditions are not well-known, and it may well be that light responses induced by shot noise require different signal processing tactics than those induced by bright light.
2 Theoretical foundation

2.1 Background

This chapter briefly covers the structure and function of the insect visual system, some problems related to night vision and how to analyse photoreceptor performance with low-intensity stimuli. A short summary of relevant methods will be presented at the end of the chapter. Although the matters related to night vision are relevant to all animals, the focus will be on the insect visual system, specifically the nocturnal American cockroach, *Periplaneta americana*.

![Fig. 1. The American cockroach, *Periplaneta americana*. Left: a cockroach recovering from CO₂ anaesthesia. Right: Close-up photo of the preparation used for the intracellular recordings with sharp microelectrodes (c.f. Materials and methods). After removal of the antennae the prothorax is mounted on an objective glass and the recording electrode is inserted into the compound eye retina through a small hole cut in the cornea.](image)

2.2 The cockroach visual system

The cockroach visual system consists of two simple eyes, *ocelli*, and two large compound eyes. Ocelli and compound eyes give input to the optic lobes, which contain three visual ganglia responsible for processing the visual information.

The ocelli of the cockroach can be recognized as two large white spots, located between the compound eyes at the base of the antennae (Cooter 1975, Weber & Renner 1976). Each ocellus contains ca. 10,000 photoreceptors that converge to only four large 2nd order lamina cells (Toh & Sagara 1984).
Consequently, the ocelli have extremely high light-sensitivity and seemingly more or less non-existent spatial resolution, which is why they are considered as general intensity level detectors incapable of forming images.

The three visual ganglia in the optic lobe (lamina, medulla and lobula) process the visual information provided by the compound eyes. The lamina receives its input from the photoreceptors, and the anatomy of the retina-lamina projection in the cockroach suggests large-scale spatial pooling (Ribi 1977). The medulla processes colour and polarization information (Kelly & Mote 1990). Unfortunately, at the moment there are no studies about the function of cockroach lobula. In other insects lobula (or lobula plate) participates in e.g. processing movement information. The best studied lobular neurons in insects are the lobula giant movement detector (LGMD) in locusts (O'Shea & Williams 1974, Rind 2002) and the lobula tangential cells in flies (Borst et al. 2010).

The optic lobe input containing all the information (colour, contrast, movement, polarization etc.) in raw form is delivered by the photoreceptors located in the retina of the ocelli and more importantly the compound eye, which shall be discussed next.

2.2.1 The compound eye

The primary visual organ in most adult insects is the compound eye. Compound eyes consist of repeating units called ommatidia, which together form the retina. The compound eye surface, or cornea, is formed by ommatidial facet lenses. Unlike in most insects, which have hexagonal and regularly distributed lenses, the lenses in cockroach compound eye vary significantly both in size and shape within the same eye (Heimonen et al. 2006). The number of ommatidia in adult cockroach ranges between ca. 3300 to 5700 between individuals, and interestingly varies substantially also between the eyes in a single specimen (Füller et al. 1989).

Based on their optical structure, insect compound eyes can be categorized in two main types: apposition and superposition type (Fig. 2 and Nilsson 1989). The cockroach has the apposition type (Butler 1973), where each ommatidium receives light from a single facet. In superposition eyes ommatidia collect light from several facets, granting high optical sensitivity but often poorer spatial resolution. Insect superposition eyes can be further classified according to their optical structure as refracting, reflecting or parabolic, where the light from several
facets is focused into a single photoreceptor by refraction or a combination of refractive and reflective optics, respectively. Superposition compound eyes are found in e.g. beetles and moths, and they are more common in nocturnal than diurnal species. A third variant of the superposition eye called the neural superposition eye is found in higher flies (Brachycera), which implement the superposition principle by neural pooling of photoreceptor signals in the 2nd order cells in the lamina.

![Schematic Diagrams](image)

**Fig. 2.** Schematic Diagrams the (A) apposition and (B) refracting superposition eye. The optical path of the light is coloured grey and the target photoreceptor black. A = aperture diameter, f = focal length, c = corneal facet lens, cc = crystalline cones, p = screening pigment, rh = rhabdom, cz = clear zone, l = rhabdom length, and d = rhabdom diameter. Reprinted from Current Biology, Vol. 14(15), Warrant EJ, Kelber A, Gislén A, Greiner B, Ribi W & Wcislo WT: Nocturnal Vision and Landmark Orientation in a Tropical Halictid Bee, 1309-1318, 2004, with permission from Elsevier.

Despite their inferior sensitivity in comparison to superposition eyes, apposition eyes are found in several nocturnal insects besides the cockroach, such as the tropical sweat-bee *Megalopta genalis* (Greiner *et al.* 2004) and the carpenter bee *Xylocopa tranquebarica* (Somanathan *et al.* 2009). In nocturnal
species with apposition eyes, several adaptations in the visual system compensate for the low optical sensitivity (Greiner 2006a).

### 2.2.2 Ommatidia and photoreceptors

Underneath the lens in each ommatidium lies a crystalline cone ensheathed by two primary pigment cells, which isolate the cones between adjacent ommatidia. The crystalline cone focuses the incoming light into light sensitive photoreceptors. Cockroach ommatidia contain eight photoreceptors, five of which are maximally sensitive to green and three to UV, with peak sensitivities at 507 nm and 370, respectively (Butler 1971).

The corneal lens at the distal end of the ommatidium focuses light into the rhabdom formed by the photoreceptors’ light-sensitive microvillar membrane structures, rhabdomeres. There are two basic types of rhabdom: open and fused. In open rhabdoms the rhabdomeres are separated by an intra-rhabdomeral space whereas fused rhabdoms have no empty space as the rhabdomeres are joined together forming a large light-sensitive structure in the ommatidium centre. The fused rhabdom is potentially capable of capturing more photons than the separated rhabdomeres in an open rhabdom. In the open rhabdom the photoreceptors have different visual fields, which is necessary for neural superposition eyes found in flies. Fused rhabdoms have worse spatial resolution than open rhabdoms, and do not allow neural superposition since all rhabdomeres collect light from the same optical axis. However, fused rhabdoms are more sensitive and potentially capable of catching more photons as there is no empty space within the rhabdom (Snyder et al. 1973). Typically for a nocturnal insect, the cockroach has a fused rhabdom formed by rhabdomeres of green- and UV-sensitive photoreceptors (Butler 1971).

From the retina photoreceptors project their axons to the 2nd order cells in either the first or the second visual neuropil, the lamina or medulla. In the cockroach, two axon classes have been described (Ribi 1977): short visual fibres (svf), which synapse in the lamina and long visual fibres (lvf) that go through lamina and connect to the medulla. The spectral sensitivity of svf and lvf are green and UV, respectively (Mote 1990). In flies, the svf and lvf fibres form the motion and colour sensitive pathways, in that order. The pathways are not completely segregated, however, since the lvf cells contribute to the motion sensitive pathway through gap junction connections with the svf axons (Wardill et al. 2012). While
the structure and function of the colour and motion sensitive pathways are documented in *Drosophila* (Morante & Desplan 2008, Morante & Desplan 2004, Rister *et al.* 2007) comparable studies have not been conducted in the cockroach.

In the cockroach, photoreceptor axons leave the retina in bundles of eight passing through two basement laminae, after which they travel in bundles of 6-20, enclosed within myelin sheaths (Ernst & Füller 1987, Ribi 1977). The 2nd order cells in lamina have wide dendritic arbours (Ribi 1977) indicating the possibility of large scale spatial pooling of photoreceptor inputs, a mechanism generally considered to benefit night vision (Greiner *et al.* 2004, Greiner *et al.* 2005, Klaus & Warrant 2009, Theobald *et al.* 2006, Warrant 1993, Warrant 1999).

### 2.3 Photoreceptor signalling

Photoreceptors transform the energy of absorbed light into an electrical signal called the photoresponse. Photoresponse shape is determined by the properties of the phototransduction cascade and the photoreceptor membrane. Phototransduction in insect photoreceptors takes place in the microvillar rhabdomere, where it creates an inward cation current, whereas the rest of the cell body acts as an electrical current-to-voltage converter that shapes the voltage response driven by the light-induced current.

Phototransduction and the electrical membrane properties of photoreceptors go hand-in-hand with the insect’s visual ecology (Gonzalez-Bellido *et al.* 2011, Laughlin & Weckström 1993, Niven *et al.* 2007). For example, insects that move quickly or need to see fast objects tend to have equally fast vision; similarly slowly moving insects that are nocturnal have slower, but more sensitive visual systems. In addition to inter-species differences in the visual system, other intraspecific specialisations have been described in several species where the males typically have eyes or eye regions with appropriate optical features for detecting and tracking female conspecifics (Hardie 1986, Zeil 1983). Along with the compound eye optics, photoreceptors may have different spectral sensitivities (Arikawa *et al.* 2005), photoresponse kinetics (Burton *et al.* 2001, Hornstein *et al.* 2000) and membrane properties (Laughlin & Weckström 1993) suitable for this task.

The optimization between the sensory system and the behaviourally relevant sensory input is called matched filtering in a very general sense. Matched filtering can be established at the interface between the sensory input from the
environment and the receptor array in the sensory system (Wehner 1987), or within the sensory system between the transduction process and the following neural processing (Laughlin 1996). Matched filtering ensures that relevant information is captured and processed efficiently by the sensory system, while noise and excess information is filtered out optimally. For a nocturnal insect such as the cockroach, a “basic” matched filter set should include a sensitive compound eye with adequate spatial resolution, followed by neural circuitry capable of extracting information from an unreliable low-intensity light input. In photoreceptors, the two stages of matched filtering are provided by the phototransduction cascade and the photoreceptor membrane.

2.3.1 Phototransduction

The photoresponse is initiated by a biochemical process called the phototransduction cascade. Most of what we know about phototransduction in insect photoreceptors comes from studies done on the fruit-fly *Drosophila melanogaster* (Hardie 2012). In *Drosophila*, phototransduction is initiated by absorption of a photon in a rhodopsin molecule, which is then photoisomerised into metarhodopsin. This triggers a G-protein mediated reaction series, which activates phospholipase C (PLC) that hydrolys a membrane lipid component called phosphatidyl-inositol (4, 5) biphosphate (PIP2) into diacylglycerol (DAG), inositol (1,4,5) triphosphate (IP3) and a proton. According to a recent study, the substitution of PIP2 by the smaller size DAG leads to a reduction of the membrane area and hence tends to increase membrane tension, causing mechanical stress, which contributes to the opening of the ion channels responsible for generating the light-induced current (Hardie & Franze 2012). The light-induced current in *Drosophila* is mediated mainly by two channel types: the transient receptor potential TRP (Hardie & Minke 1992, Montell & Rubin 1989) and the transient receptor potential like TRPL (Niemeyer *et al.* 1996, Phillips *et al.* 1992), which have different biophysical properties (Reuss *et al.* 1997) and are active in different intensity regimes (Bähner *et al.* 2002). TRP has small single channel conductance and mediates responses mainly in bright light, while the single channel conductance of TRPL is larger than TRP and the channel is activated mostly in low-light intensities. During prolonged light stimulation TRPL channels are translocated from the microvilli to the cell body, contributing
to long-term light adaptation (Bähner et al. 2002). The photoresponses in light-adapted photoreceptors are thus mediated mainly by TRP channels.

*Drosophila* can hardly be assumed to represent all insects and the phototransduction mechanisms are likely to vary among different species. For example, in the cockroach the light-induced current seems to be mediated mainly by a single channel type with similar properties to the *Drosophila* TRPL (Immonen 2013). Unfortunately, data on the phototransduction cascades in other insects are lacking at present.

Phototransduction culminates in the opening of light-dependent ion channels. The conductance change caused by channel opening leads to an inflow of Ca$^{2+}$ and Na$^+$, which generates an electrical current and voltage change in the photoreceptor membrane. Absorption of a photon in a microvillus produces a microscopic single photon response called a (quantum) bump. With a bright stimulus several bumps from different microvilli sum up to form a noisy macroscopic light response. The lower the rate of photon absorptions, the noisier the response is. With increasing intensity the noise decreases since the relative proportion of shot noise in the signals declines and bumps become smaller due to light adaptation. During prolonged constant intensity light stimulation the photoreceptor adapts, which involves negative feedback regulation at various stages in phototransduction, and responses become two-phased, with an initial transient that decays to a plateau depolarization level.

Bump current responses in voltage-clamped cells are in the range of tens of picoamperes and macroscopic current responses can transiently reach tens of nanoamperes, when the stimulus is given after a dark-adaptation period. However, the light induced current (LIC) in voltage-clamp experiments is driven by a practically constant driving force, determined by the holding potential $V_{hold}$ and the LIC reversal potential $E_{LIC}$: $I = g_{LIGHT}(t)(V_{hold} - E_{LIC})$. Thus the current is necessarily different in voltage-clamp than during physiological conditions *in natura* or in current clamp recordings, where the voltage can freely change and light responses are limited by the photoreceptor’s resting potential and $E_{LIC}$. Cockroach photoreceptors are normally limited by a resting potential of ca. -70 mV and the $E_{LIC}$ of ca. +10 mV (Salmela et al. 2012).

Voltage bump amplitude in cockroach photoreceptors varies from a few to ca. ten millivolts. Macroscopic impulse responses and initial transients for prolonged stimuli have maximum amplitude of ca. 60 mV, but as light-adaptation takes effect the voltage tends to decay to a plateau level. When compared to other
species the adaptation kinetics and the plateau level are highly variable in cockroach photoreceptors (Heimonen et al. 2006).

The amplitude and kinetics of the voltage response to light are governed by the light-dependent conductance and the membrane impedance, both of which are typically high in nocturnal insects. The impedance is determined by the photoreceptor’s electrical properties, which shall be discussed next.

2.3.2 Photoreceptor membrane

The photoreceptor membrane is formed by a lipid bilayer, which is formed by the highly convoluted microvilli in the light-sensitive rhabdomere and the relatively smooth part in the rest of the soma. The ion concentration gradient between the intra- and extracellular volumes creates a trans-membrane voltage, which the photoreceptor can modulate to transmit electrical signals. Ion traffic between extra- and intracellular spaces can be regulated by ion channels and transporters (pumps, exchangers, etc.). Together membrane capacitance and ion channel net conductance define the membrane impedance.

Membrane impedance (or total cell impedance) controls how a current flow through the membrane is transformed into a voltage change. The impedance is determined by the membrane properties of capacitance (C) and resistance (R). Capacitance is a property of the lipid bilayer, which functions as an insulator similar to electrical capacitors. Resistance is determined by permeability of the membrane to solute ions, essentially by the probability of ion channels being open in the membrane.

The impedance is formed by membrane capacitance (C) and resistance (R), which form a low-pass filter circuit with cut-off frequency $f_c = 1/(2\pi\tau)$, where $\tau$ is the membrane time constant $\tau = RC$. The membrane time constant describes how fast the current that passes through the ion channels, and thereby through the membrane, is converted into a membrane voltage change. By modifying either resistance R or capacitance C, the membrane time constant could be regulated by the cell to allow faster voltage changes or limiting the responses to slow signals. Membrane capacitance is relatively constant, although small variation may occur due to e.g. membrane shedding with circadian rhythm (Williams 1983). Conversely, the resistance could be dynamically adjusted by opening and closing ion channels.
Ion channels that are permanently open and allow constant flow of ions are called passive or leak channels (Hille 2001). Ion channels may also be gated by some biophysical property, which controls the channel’s conductance by opening and closing of the channel pore. Gating can be controlled by a biochemical ligand (ligand-gated channels), the trans-membrane potential difference (voltage-gated channels) or a physical signal such as mechanical stimulation (transduction channels in sensory cells). In addition to the light-gated channels opened by the phototransduction cascade, the most important determinants of membrane resistance in insect photoreceptors are the voltage-gated channels.

**Voltage-gated potassium (Kv) channels**

Voltage-dependent potassium (Kv) channels are the most important light-independent channel type in insect photoreceptors, where their primary function is to adjust membrane gain and kinetics during light responses. The Kv channel types relevant for insect photoreceptors can be roughly placed within two categories: sustained non-inactivating conductances reminiscent of the delayed rectifier conductance in spiking neurons, and transiently activated, that is, inactivating conductances, often referred to as A-type conductances. Expression of specific types of Kv channels in insect photoreceptors has been shown to be linked to the visual ecology of the species in question. Comparative studies of Kv channel expression in fly photoreceptors (Laughlin & Weckström 1993) have shown that nocturnal or crepuscular slow-flying species express transient inactivating Kv conductances, whereas diurnal fast-flying species express sustained non-inactivating Kv conductances.

One can propose a scheme for Kv channel function in depolarizing insect photoreceptors, following Laughlin & Weckström (1993). Animals that are normally active in dim illumination can prevent saturation of their photoresponses by sudden bright light by activating a transient Kv conductance. The following inactivation restores the high membrane impedance, which amplifies the light-induced currents into large voltage responses. But while amplification increases the photoreceptor’s sensitivity it also leads to temporal pooling, i.e. low-pass filtering of the response, due to the longer membrane time constant caused by the increased resistance. Since slow-flying nocturnal or crepuscular flies do not need fast vision the decreased signalling speed may not be an important issue for them. Moreover, because transient Kv channels inactivate and thus pass K⁺ ions only
briefly, the $K^+$ concentrations over the membrane can be restored by the Na-K ATPase with lower metabolic costs compared to a situation, when the channels would remain open. In *Drosophila* photoreceptors the nonlinear behaviour resulting from the inactivation of Shaker conductance also improves information coding (Juusola et al. 2003, Niven et al. 2003a, Niven et al. 2004).

Contrary to their crepuscular relatives, diurnal flies tend to have a dominating sustained $K_v$ conductance. The channels open during light-induced depolarization of the membrane, decreasing the membrane time constant and thus allowing generation of faster voltage responses. The open channels also decrease the membrane gain and therefore contribute to the net light-adaptation required for preventing saturation of the photoresponses. The increased signalling speed and lower membrane gain provided by sustained $K_v$ conductance are vital for flies manoeuvring their flight under daylight intensities. On the other hand, the use of sustained activation of $K_v$ channels has the detriment of vastly increasing the energy consumption, because of the large movement of ions during the photoresponses and the need to restore the membrane balance by the Na-K-ATPase (Niven et al. 2007, Niven et al. 2003b).

*Other voltage-gated channels and membrane electric mechanisms*

Besides $K_v$ conductances, several other voltage-gated conductances have been found in photoreceptors of various insect species.

Honeybee drone photoreceptors express a sodium conductance, which accelerates the rising phase of photoresponses and amplifies voltage responses to small contrasts, such as the honeybee queen against a bright sky backdrop (Coles & Schneider-Picard 1989, Coles & Schneider-Picard 1989). The sodium conductance causes dark-adapted cells to spike and small-amplitude spikes can be recorded from worker bees as well (Coles & Schneider-Picard 1989). Interestingly, photoreceptors of some nocturnal insects such as *Megalopta* (Berry et al. 2011) and *Periplaneta americana* (Weckström et al. 1993) use action potentials in addition to the graded potential signal, but the identity of the ionic conductance behind spiking has not been studied in either species, although the time-course – the speed – of the spiking signals suggests a sodium channel based mechanism.

Cacophony is a voltage-dependent calcium channel that mediates the synaptic release in the *Drosophila* photoreceptor axon terminals (Astorga et al. 2012).
While Cacophony is unlikely to have a role in signal processing in the photoreceptor soma, its activation may be seen in some photoreceptors with short axons, e.g. *Drosophila*, where the feedback mechanisms in the 1st neuropil, the lamina, seem also to change the responses in the soma (Nikolaev *et al.* 2009, Zheng *et al.* 2009).

Voltage-gated chloride conductances have been reported in *Drosophila* photoreceptors, but their role in photoreceptor function is not clear (Ugarte *et al.* 2005). The chloride conductance in *Drosophila* photoreceptors resembles the CLC-2 channel found in several neurons, where it serves various functions related e.g. to regulating cell volume, resting potential, etc. (Jentsch *et al.* 1999).

Other electrical properties found in insect photoreceptors include pumps, transporters and exchangers, which are required to keep the photoreceptors in homeostasis (Gerster 1996, Gerster *et al.* 1997, Gerster 1997, Jansonius 1990, Uusitalo & Weckström 2000). However, the role of such processes for signalling is far less understood than for ion channels, although they are bound to be always present as a slow background signalling pathway. By definition, the Na-K pump is hyperpolarizing and the Na/Ca-exchanger depolarizing, and their overlapping influence on the photoreceptor membrane potential is bound to form a complex balance with the ion channels in defining the membrane depolarization during long-lasting light stimulation.

### 2.3.3 Light- and dark-adaptation

Photoreceptors must encode a wide range of light intensities into a voltage range limited to tens of millivolts to make use of the full intensity range effectively. To establish this, photoreceptors need to adapt to the prevailing illumination. In light- and dark-adaptation the gain and sensitivity of photoreceptors are adjusted in order to maximize the information transfer at the given illumination (Juusola *et al.* 1994, Juusola & Hardie 2001, Laughlin & Hardie 1978, Laughlin 1989). This usually results in a trade-off both between the gain and temporal acuity as well as the sensitivity and spatial acuity of the photoreceptor. These trade-offs can be recognized in both the dynamic light/dark-adaptation processes during light stimulation as well as in the permanent anatomical and physiological adaptations related to the circadian activity of the species (Warrant 2001).

Light responses elicited after a long enough dark-adaptation period exhibit short-term adaptive behaviour, where typically the voltage drops a little after the
initial transient and settles at a plateau level when stimulated with a light pulse of constant intensity. Most of our knowledge about light and dark adaptation in insect photoreceptors comes from studies of flies of various related species of the genera *Calliphora*, *Musca* and *Drosophila*. Although the insects vary immensely in their habitats and ways of life, it has generally been assumed that the fly prototype is more or less valid. One counter-example is the light adaptation in cockroach photoreceptors, which appears to be very different from most other studied insects. While the photoresponses in e.g. flies adapt to a steady-state plateau depolarization within tens of milliseconds, cockroach photoreceptors adapt with varying speed and may adapt very little or even back to a state, which is reminiscent of dark-adaptation, with distinguishable single photon responses despite a bright saturating light stimulus being present all the time (Heimonen et al. 2006).

During light-adaptation the photoresponse gain and the sensitivity in terms of photon catch are modified. Photon catch in apposition eyes is known to be adjusted with several mechanisms that modify the optical properties of the photoreceptors (Nilsson 1989). A pupil mechanism can be implemented by moving the primary pigment cells around the crystalline cone, which effectively changes the photoreceptor’s aperture and acceptance angle, or by moving screening pigment cells around the rhabdom, which has a lesser effect on the acceptance angle. Both types of pupil mechanisms lower the photon catch in bright light as the pigments prevent photons from reaching the rhabdom. The rhabdom diameter can also be varied, as has been reported to occur in e.g. locust photoreceptors (Williams 1983, Williams 1982), where light adapted small diameter rhabdons absorb fewer photons than dark-adapted large diameter rhabdons. Changes in photoresponse gain are implemented by both the phototransduction cascade and the photo-insensitive photoreceptor membrane. Light-adapted bumps are smaller and faster than dark-adapted bumps, which enables generation of responses with improved temporal dynamics without saturation in bright light, thus allowing higher information transfer rates (Juusola & Hardie 2001, Juusola & de Polavieja 2003). In *Drosophila*, translocation of the light-sensitive high-conductance TRPL channels from the microvilli to the soma occurs during light-adaptation (Bähner et al. 2002). Phototransduction efficiency in terms of transduced photons is lowered since after bump generation the microvilli have a brief refractory period and cannot generate a new response for a short while. This kind of mechanism ensures that there are always microvilli in
reserve available for photon absorption even during bright intensity stimulation (Song et al. 2012). The photo-insensitive membrane of the photoreceptor soma contributes to the gain regulation through voltage-dependent impedance regulation, as $K_v$ conductances activated by the light-induced depolarization decrease the gain and increase the temporal cut-off frequency of the membrane impedance (Weckström et al. 1991).

In contrast to light-adaptation, dark-adaptation tunes the photoreceptors to optimize their performance under low-light illumination. In principle the dark-adaption processes are largely the opposite of light-adaptation mechanisms. The pupil is opened, transduction gain is maximized and membrane gain at the dark-resting potential is large since $K_v$ channels are closed, although the speed of the changes is likely to be slower than in light adaptation. These features allow a large sensitivity and gain of photoresponses, at the cost of loss of both spatial and temporal resolution. The spatiotemporal pooling is an important feature of functional night vision, as discussed in the next chapter.

2.4 Night vision

Many insects are capable of visually guided behaviour at night (Warrant & Dacke 2010, Warrant & Dacke 2011), and even have functional colour vision (Kelber et al. 2002) and an accurate polarization sense (Dacke et al. 2003a, Dacke et al. 2003b), proving that the physical and physiological noise constraints related to night vision can be overcome by adaptations in the visual system. Visual systems of nocturnal animals have to be sensitive enough to pick up scarce photons and they must be able to process the already unreliable shot noise signals without adding too much noise on top. The main tactics for seeing at night incorporate spatiotemporal pooling of the visual input (Warrant 2006).

Evidence for functional night vision at shot noise intensities has been found using behavioural, experimental and theoretical tools from several insects. For example, photoreceptors of the housefly $Lucilia$ capture less than two photons per second at the optomotor response threshold (Dubs et al. 1981). The nocturnal bee $Megalopta$ finds its home nest flying under the canopy, when only ca. 5 photons per second are absorbed by each photoreceptor (Warrant et al. 2004). From non-insect invertebrates, the shore crab $Leptograpsus$ can follow the movement point source (a 0.5 magnitude star), when a single photoreceptor sees one photon every 3 seconds and the whole retina receives 18 photons per second (Doujak 1985).
seems therefore plausible to assume that behaviourally relevant night vision at the photoreceptor level is based on photon counting, rather than contrast coding. Photon counting requires that photoreceptors are able to capture photons and process the signal efficiently. Signal processing in night vision is essentially limited by noise, which results from the random properties of photons and the photoreceptor processes. In vertebrate eyes the responses of rods are increasingly pooled in dim light, which seems to comprise a very different method for night vision, but is of course akin to the spatial summation that may take place in further neural processing in many nocturnal insects.

2.4.1 Night vision is limited by noise

Visual systems have to cope with various types of noise that corrupt the signals and decrease their reliability as information carriers (Faisal et al. 2008). In photoreceptors the noise can be extrinsic, i.e. noise included in the light input, or intrinsic, i.e. generated within the photoreceptor itself.

Transmission of signals into the 2nd order cells in the lamina or medulla is limited by noise, which is in part external due to the noisiness of captured light and in part internal, because the phototransduction and signal conduction on the membrane and over synapses are stochastic processes (Laughlin 1990, Laughlin 1981). While noise poses a problem for all communication, it is critical for information transfer mediated by scarce single photon events, i.e. bumps, which need to propagate to the 2nd order neurons as reliably as possible in order to enable functional night vision. Insect photoreceptors constitute an ideal system for studying the effects of noise in the early visual system since they can be studied both in vivo and in vitro with various electrophysiological methods, which allow the separation of different noise sources and estimation of the information transfer by e.g. measuring the signal power relative to noise power, i.e. the signal-to-noise-ratio (SNR).

Extrinsic noise in the visual system results from the random properties of discrete photons emitted or reflected by objects, which contribute to so-called shot noise. The rate of photons emitted by a light source follows a Poisson distribution, where the variance of rate is equal to the mean rate. The variability of the photon rate is called shot noise. The signal-to-noise ratio (SNR) for a Poisson process is \( \text{SNR} = \sqrt{N} \), where \( N \) is the mean number of photons within a time window. Shot noise is therefore most significant at low-light levels, where
the SNR and mean photon rate are small, but its effects decrease logarithmically
with increasing light intensity.

Transduction noise is generated by the stochastic biochemical processes
within the phototransduction cascade, which result in a variable end product, i.e.
the single photon response. Transduction noise makes the bumps vary in timing,
size and shape. In insect photoreceptors, latency and amplitude variability are the
most important components of phototransduction noise (Henderson et al. 2000,
Laughlin & Lillywhite 1982).

Dark noise or thermal noise in photoreceptors refers to generation of “false”
responses when no photons are absorbed in the photopigment. Dark noise is
caused by thermal or spontaneous isomerization of rhodopsin, which triggers the
phototransduction cascade. Because spontaneous bumps are indistinguishable
from actual bumps the effect of dark noise is largest in dim light, where
photoreceptors are counting photons. While detectable in vertebrate rods with
long-wavelength rhodopsins, which require the lowest energy for isomerization,
dark noise is rare in insects and e.g. locust photoreceptors produce less than ten
spontaneous bumps per hour (Laughlin & Lillywhite 1982). However, dark noise
resulting from spontaneous activation of intermediate transduction steps such as
G proteins may occur, generating small bump-like noise events (Burton 2006,
Chu et al. 2013, Hardie et al. 2002).

Neural noise is generated by the electrical and biochemical processes, which
both shape the responses arising from the conductances activated during sensory
transduction and transmit the signals along the visual pathway within the cell
membrane and between adjacent cells (White et al. 2000). For example, the
activity of ion channels and the release and reception of synaptic vesicles are
random events, which result in noise. In graded signals noise is manifested in a
stochastic fluttering of the membrane voltage. In neurons that transmit
information with action potentials noise causes jittering in the spike timing.

Although usually considered harmful for signalling, noise can sometimes
have positive effects and actually improve the information coding of neurons. In
spiking neurons the random fluctuation of membrane voltage may initiate action
potentials for an otherwise sub-threshold input in a phenomenon referred to as
stochastic resonance (Collins et al. 1995, Wiesenfeld & Moss 1995). While
photoreceptors in both cockroach compound eye and Megalopta ocelli employ
spikes, whether they utilize stochastic resonance to enhance night vision is not
known at the moment.
Most systems analyses and information theoretical tools assume additive noise that is independent from the signal, but in fact most of the noise sources in neurons are multiplicative and often largely dependent on the signal. The problem of multiplicative noise in photoreceptors of nocturnal animals is apparent in shot noise, which can be considered as both noise and signal at the same time. It is this “noise” which is multiplied by the noisy membrane into a voltage response. Shot noise–induced responses thus cannot be analysed by methods that assume the noise is both additive and independent from the signal.

2.4.2 Night vision requires adaptations in the visual system

For a visual system to operate in low-light intensity levels, it needs to adjust to the prevailing low-intensity light input. Comparative studies in closely related nocturnal and diurnal insects have revealed some of the features that can be tuned to improve the visual system’s performance in low-light conditions (Frederiksen & Warrant 2008b, Greiner 2006b, Greiner et al. 2004, Laughlin & Weckström 1993, Somanathan et al. 2009, Warrant et al. 2004). In general, the adaptations involve spatiotemporal summation of the visual input, i.e. collection of more photons by sampling a larger area for a longer time, which simultaneously increases the sensitivity and decreases the spatiotemporal resolution. Next I will shortly describe the most important features responsible for spatiotemporal filtering in the visual systems of nocturnal insects.

Anatomical and optical adaptations for night vision aim to increase the light-sensitivity of the eye. The obvious adaptation is to have larger compound eyes and larger lenses, which allow collection of more photons from a larger area. Sensitivity can also be improved by implementing superposition optics and larger rhabdoms, which increases the number of photons captured in the microvilli. As a general “rule”, nocturnal insects have larger eyes, lenses and rhabdoms than their diurnal relatives. However, despite the greater sensitivity of the superposition compound eye, several nocturnal insects have apposition eyes (Greiner 2006a).

The gain of both the phototransduction cascade and the photoreceptor membrane (i.e. the impedance) in nocturnal insects should be high to enable generation of large single photon responses. Although there is very little published data available on phototransduction besides those in *Drosophila melanogaster*, nocturnal insects, based on voltage bumps, have slower photoresponses and larger single photon responses than diurnal insects (Faivre &
Juusola 2008, Frederiksen et al. 2008). These features are obtained by a combination of slow phototransduction and high membrane impedance with strong low-pass properties. Together, phototransduction and membrane gain turn the photoreceptor into an efficient low-pass filter that promotes temporal pooling of the light stimulus. But while temporal pooling increases the sensitivity of the visual system, it decreases the temporal resolution and faster signals are lost due to the filtering.

Vision with single photon responses could be enhanced by employing a voltage-sensitive amplification of the bumps. Interestingly, cockroach photoreceptors employ action potentials in their axons, and in vivo recordings conducted in the photoreceptor soma reveal an attenuated spikelet (Weckström et al. 1993). Spikes are also utilized in the ocellar photoreceptors of Megalopta (Berry et al. 2011). In both Periplaneta and Megalopta, the sensitivity of photoreceptors varies considerably. The spikes may be related to preventing attenuation during the long distance the signals have to travel before reaching the synaptic terminal. Action potential coding combined to the net variability of photoreceptor function has been shown to facilitate a sort of population code, where the variable graded responses are coded into APs, and the 2nd order cells receiving the axons pool the signals and are able to achieve larger information rates that are higher than from pooling identical signals (Heimonen et al. 2006).

Neuroanatomical adaptations in night vision incorporate spatiotemporal pooling by neural connections between photoreceptor axons and laterally branching dendrites of large monopolar cells (LMC) in the 1st visual neuropil, Lamina. Branching has been reported in e.g. the cockroach (Ribi 1977) and nocturnal bee Megalopta (Greiner et al. 2004, Greiner et al. 2005). Theoretical studies based on LMC anatomy have shown that spatiotemporal pooling in LMCs could significantly improve the sensitivity of the visual system (Klaus & Warrant 2009, Theobald et al. 2006, Warrant et al. 1996). However, no electrophysiological studies of spatiotemporal pooling in the lamina of any nocturnal insect have been published.

In addition to the anatomical and physiological adaptations described above, temporal pooling can be accomplished behaviourally by adjusting the speed of the optic flow sampled by eyes through locomotion. Moving slowly and looking at objects a little bit longer gives the visual system time to collect more photons. This kind of temporal pooling has been found in e.g. nocturnal bees and ants, which move slower than their diurnal relatives or conspecific caste members to
allow their visual system to collect enough photons for guiding their locomotion (Baird et al. 2011, Narendra et al. 2011).

2.4.3 Quantifying the night vision performance of photoreceptors

The signalling performance of photoreceptors can be studied experimentally by analysing the responses elicited by low-intensity stimuli. Theoretical approaches can be used to estimate the sensitivity and photon catch of photoreceptors.

Pulse responses

Pulse responses give information about the sensitivity and kinetics of light responses. Temporal pooling, i.e. integration of the light input via the phototransduction cascade and the membrane impedance, can be evaluated from the kinetics of low-intensity light pulse responses. A commonly used method is to measure the integration time, i.e. half-width of a low-intensity impulse response (Aho et al. 1988, Frederiksen et al. 2008, Skorupski & Chittka 2010, Warrant 1999).

The randomness of photons and phototransduction causes variable light responses. Laughlin and Lillywhite studied the contribution of shot noise and phototransduction noise to the variability of light responses with dim pulses (Laughlin & Lillywhite 1982). Hornstein et al. (Hornstein et al. 1999) used receiver operating characteristics (ROC) analysis (Cohn 1977) to estimate the reliability of low-light signalling by locust photoreceptors. Both groups concluded that in dim light photoreceptors operate close to the theoretical upper limit set by the stochastic properties of light, suggesting that the role of phototransduction noise is insignificant compared to shot noise.

Although by using impulse and step responses one can assess various properties related to the reliability of signalling in dim light, these methods do not tell us how much information can actually be coded by the photoreponses. For this task, photoreceptors must be analysed using dynamic noise stimuli, which allow systems identification and information theoretical analyses.
**Dynamic noise stimuli**

Transfer function analyses (Juusola et al. 1994, Kouvalainen et al. 1994) have become the standard tool for estimating the information processing capability of insect photoreceptors.

Signal-to-noise-ratio (SNR) estimation is a common method for analysing signalling performance in photoreceptors (Kouvalainen et al. 1994). When measuring SNR, multiple responses to identical pseudorandom stimuli are recorded and averaged, and the average represents the signal and the noise component is the difference between the average and a single trial. SNR in the frequency domain is then obtained as the ratio of the power spectra of signal and noise: \[ \text{SNR}(f) = \frac{P_S(f)}{P_N(f)} \]. However, estimating SNR with white noise modulated stimuli may bias the results, if the stimulation used has a broad bandwidth that leads to a low contrast at those frequencies that the photoreceptors normally respond to. To compensate for this, the stimulus bandwidth has to be narrowed or, alternatively, some other type of noise modulation used, for example 1/f noise or some form of “naturalistic” signals with similar properties.

While measuring the signal component by averaging works well for intensities that elicit graded responses, it does not suit shot noise responses. This is because averaging removes random components, i.e. the single photon responses generated by the low-intensity stimulus. For nocturnal insects shot noise is an important and sometimes the only information carrying signal component, and will be lost due to averaging. Although shot noise is generated by a random process, its statistics are controlled by the ambient illumination and reflectance of the objects in the visual field. Shot noise is thus bound to contain some, albeit very little amount of information about these properties. However small the information is, it is sufficient for behavioural control for nocturnal insects. Therefore, a method that can assess the information content of shot noise is necessary for studying the coding performance of photoreceptors adapted for night vision at their natural preferred intensity level.

**Theoretical approaches**

Sometimes the experimental assessment of photoreceptor sensitivity is not feasible due to e.g. experimental difficulties. With adequate knowledge about the optics, geometry and spectral sensitivity of the photoreceptor, along with spectral
content of the illumination, one can estimate the sensitivity and photon catch of photoreceptors, with outcomes close to experimentally derived results (Frederiksen & Warrant 2008a, Land 1981).

The integration time obtained from dim intensity pulse responses can be used to calculate an estimate of the photon catch under different intensities when enough information about the optics and geometry of the photoreceptors as well as the spectral properties of the illumination and the sensitivity are available. The sensitivity of the photoreceptors in terms of captured photons within the integration time can be estimated by e.g. the Land equation (Land 1981, Warrant & Nilsson 1998, Warrant 1999). In the nocturnal bee *Megalopta*, the catch during an integration time of 32 ms is 0.15 photons, translating to ca. 4.7 photons per second (Warrant et al. 2004). However, even this might be an overestimate since the equation does not take into account the number of photoreceptors within an ommatidium, as the incoming photons could potentially be absorbed by any of the eight photoreceptors, assuming the photon’s wavelength matches the photoreceptors’ spectral sensitivities.
3 Aims

In a broad sense, the general aim of this thesis was to study how vision in dim light works in cockroach photoreceptors by investigating the properties of low-intensity light and the biophysical and signalling properties of the photoreceptors.

Due to the random nature of photons, the low intensity illumination nocturnal insects are subject to forms the first information bottle neck in vision. Once the energy of the photons has been converted into a conductance change by the phototransduction process, the following signal processing is governed mainly by the electrical properties of the cell membrane, although light- and dark-adaptation processes will dynamically modify the photoreceptor performance with varying illumination.

The aims of this work were thus to characterise:

1) the biophysical properties of the photoreceptors
2) the signalling and adaptation dynamics of photoresponses
3) the information content of low-intensity contrast stimuli and how much of that information cockroach photoreceptors can encode into light responses
4 Materials and methods

This chapter covers the methods used in this thesis work briefly, more detailed descriptions can be found in the original articles (I, II, III).

4.1 Experiments

All experiments were conducted on adult male cockroaches (*Periplaneta americana*). Photoreceptors were studied using both *in vivo* intracellular recordings with sharp microelectrodes and *in vitro* whole-cell patch-clamp from isolated ommatidia. All recordings were done in room temperature (20-25 °C) from green sensitive cells, identified by their response to stimulation with a green LED.

4.1.1 In vivo intracellular recordings with sharp microelectrodes

The cockroach was anesthetized using carbon monoxide. The thorax was attached to an objective glass using a beeswax/resin compound. After jaw removal a cut was made to the frontal head to prevent mechanical disturbance from muscle activity. Holes for the electrodes were cut in the cornea using a razor blade and covered with grease to prevent drying. A silver wire reference electrode was inserted to the other eye.

Recordings were done using borosilicate capillary electrodes filled with 2M KCl, electrode resistances were between 50 MΩ and 150 MΩ in tissue. Electrode signals were amplified with npi-5s single electrode intracellular amplifier (npi electronics GmbH, Tamm, Germany) in discontinuous current clamp mode. Light stimuli were delivered with a 525 nm green LED through a light guide directed to the eye with a cardan arm positioning system.

4.1.2 In vitro whole-cell voltage-clamp recordings from isolated ommatidia

Data from patch-clamp experiments are courtesy of Stephan and Yani Krause, Roman Frolov and Esa-Ville Immonen. Whole-cell voltage clamp recordings were used to obtain the parameters required for the photoreceptor models. Steady-
state conductances and kinetics for activation and inactivation of the voltage-
dependent conductances were recorded with voltage-clamp pulse protocols.

Macroscopic light responses to a naturalistic contrast stimulus taken from the
van Hateren database (van Hateren 1997) were recorded in voltage-clamp. Single
photon responses were recorded by stimulating the photoreceptors with a short
pulse, which on average caused absorption of less than one photon. Response
latency and amplitude were analysed offline.

4.2 Modelling

The electrical properties and phototransduction properties obtained from the
whole-cell patch-clamp experiments were inserted into a mathematical model.
The model was based on modified Hodgkin-Huxley type formalism (Yamada et
al. 1998), where the behaviour of membrane voltage is described using a group of
ordinary differential equations (Eq. 1). The model included the whole-cell
capacitance, two voltage-dependent potassium conductances (KDR and KA), a
non-specific leak conductance (gL) and the light-induced conductance gLIGHT (Fig.
3). The activation and inactivation of voltage-dependent potassium conductances
were described with differential equations based on the steady-state voltage-
dependence and kinetics (Eqs. 2 & 3). The voltage-dependent behaviour was
validated by simulating the voltage-clamp protocols used to obtain the activation
and inactivation parameters of the Kv conductances.

\[
\begin{align*}
\frac{dV}{dt} &= -(LIC + I_{KDR} + I_{KA} + I_{LEAK}) \cdot \frac{1}{C} \\
&= g_{LIGHT}(t)(V-E_{LIC}) - G_{KDR}(V-E_{K}) - G_{KA}(a_{KA})^2 i_{KA}(V-E_{K}) - g_{L}V \\
da_{KDR} &= a_{in,KDR}(V) - a_{out,KDR} \\
da_{KA} &= a_{in,KA}(V) - a_{out,KA} \\
di_{KA} &= i_{in,KA}(V) - i_{out,KA} \\
\end{align*}
\]

(1)
\[ K_{V = \text{act/inact}}(V) = \left( 1 + e^{\frac{(V - V_{50})}{\text{slope}}} \right)^P \]  

(2)

\[ \tau_k = \text{act/inact} = \frac{1}{\alpha e^{\text{slope}V} + \beta e^{\text{slope}V} + \tau_0} \]  

(3)

Fig. 3. Circuit diagram of the Hodgkin-Huxley-like model. \( V_m \) = membrane potential; C = whole cell capacitance; \( g_L \) = leak conductance; \( g_{KDR} \) and \( g_{KA} \) = the voltage-dependent potassium conductances; \( E_K \) = reversal potential of the potassium currents (-68 mV); \( g_{LIGHT} \) = light-induced conductance and \( E_{LIGHT} \) = reversal potential of the light-induced current.

Table 1. Model parameters for steady-state activation and inactivation functions (Eq. 2) and time constant (Eq. 3): \( G \) = maximum conductance, \( V_{50} \) = half activation/inactivation voltage, \( \text{slope} \) = slope factors for the steady-state parameters (Eq. 2) or time constant (Eq. 3), \( P \) = order for the steady-state parameter (Eq. 3), \( \alpha \) and \( \beta \) = activation and deactivation rates for Eq. 3, \( \tau_0 \) = offset for bell function (Eq. 3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( G ) (nS)</th>
<th>( V_{50} ) (mV)</th>
<th>( \text{slope} ) (mV)</th>
<th>( P )</th>
<th>( \alpha ) (s(^{-1}))</th>
<th>( \beta ) (s(^{-1}))</th>
<th>( \tau_0 ) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_{KDR} )</td>
<td>78</td>
<td>-31</td>
<td>12.0</td>
<td>1</td>
<td>4</td>
<td>43</td>
<td>156</td>
</tr>
<tr>
<td>( a_{KA} )</td>
<td>36</td>
<td>-43</td>
<td>8.4</td>
<td>2</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>( i_{KA} )</td>
<td>*</td>
<td>-85</td>
<td>-11.3</td>
<td>1</td>
<td>341</td>
<td>-44</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*) KA activation time constant was fixed at 1.5 ms.
A simplified photoreceptor model with stochastic light input and phototransduction was used to study the effect of shot noise and transduction noise photoreceptor information transfer properties (Fig. 4). Shot noise was modelled by generating random numbers from a Poisson distribution, whose mean was controlled by a contrast waveform, \( \lambda(t) \), in 1 ms time steps. Contrast waveforms were GWN and Gaussian 1/f with mean intensities ranging from 1 to \( 10^5 \) photons per second. The random numbers that corresponded to the number of “photons” within a bin were transformed into shot noise by convolving each “photon” with an experimentally obtained average bump waveform. Transduction noise was added by taking the bump latency and amplitude from random distributions fitted to the amplitude and latency values determined from recorded bumps. The resulting conductance, containing only shot noise or shot noise combined with latency noise or amplitude noise or both, was fed to a Hodgkin-Huxley-type model of the photoreceptor membrane. The membrane model was similar to the one described with Equation. 1 and Figure 3, with the exception of including only the KDR conductance and omitting the KA conductance completely. Solving the differential equation group with the conductance input gave a voltage response to a noisy light input. Information rates for GWN and 1/f shot noise were calculated with the classical Shannon information (Shannon 1949) and a general information rate estimator (Takalo et al. 2011).
Fig. 4. The shot-noise driven photoreceptor model used for studying the stochastic properties of low-intensity stimuli and phototransduction. The contrast waveform was used to feed a Poisson random number generator, which outputs the “photons”. The photons were transformed into shot noise by a constant bump shape filter (red). Phototransduction noise was simulated by a stochastic bump filter, whose amplitude and latency varied. The shot noise and transduction noise signals were transformed into voltage responses with a Hodgkin-Huxley type model of the photoreceptor membrane.
4.3 Analysis

4.3.1 Transfer function analyses using noise stimuli

The signalling performance of photoreceptors is often analysed by recording responses to pseudorandom noise stimuli. The method allows e.g. determination of the frequency response (i.e. gain and phase), signal-to-noise-ratio (Juusola et al. 1994, Kouvalainen et al. 1994), and impedance function (Weckström et al. 1992).

The amplitude portion of the frequency response, often referred to as the contrast gain function, tells the gain of voltage light responses in terms of millivolts per unit contrast. The phase part of the frequency response tells the phase lag induced by the photoreceptor. Phase lag is induced by the phototransduction and the membrane low-pass filter properties.

Transfer function analyses assume that the system being studied is linear, time-invariant and noise in the system is independent from the signal. While the first two assumptions can be made with proper design of the recording protocol, the latter, i.e. the independence of signal and noise is more difficult to make as discussed in the previous chapter. In light responses shot noise contributes a significant portion of the net noise. Transduction noise, i.e. the variable shape and latency of bump conductances, will increase the noise in the photoreceptor. Both shot noise and transduction noise will be multiplied while propagating through the system. The issue with noise is severe with low-intensity light stimuli, which consist of shot noise. The contrast of shot noise stimuli becomes difficult to define, since the customary definition of dynamic light contrast as the ratio of standard deviation and mean of the intensity is no longer possible to delineate.

Shannon information rate

Coherence function (Eq. 5) was calculated from the cross power spectrum \( P_{xy} \) and power spectra of the light input \( P_{xx} \) and voltage response \( P_{yy} \).

\[
\gamma^2(f) = \frac{P_{xy}^2(f)}{P_{xx}(f) \cdot P_{yy}(f)}
\]

(5)
For a noisy communication channel, the Shannon information rate (Shannon 1949) (Eq. 6) gives the transfer rate in bits/s. The methods assumes that 1) the system is linear 2) both the signal and the noise in the system are Gaussian, independent from each other and 3) that noise is additive. The Shannon information rate estimate has been applied in photoreceptor performance analyses using GWN stimuli. GWN light stimulus can linearize the photoresponse, providing that the photoreceptor is allowed to light-adapt to the mean intensity and the voltage modulation induced by the contrast changes are small enough to not induce dynamic modulation of voltage-dependent conductances.

\[ C = \int_{0}^{88 \text{ Hz}} \log_2 \left(1 - \gamma^2(f)\right) df \]  

(6)

**Non-Shannon information rate estimation**

Since the assumptions required for Shannon rate estimation are not always fulfilled, information theoretical tools requiring fewer assumptions about the nature of the signal and the noise have been developed. The information rates for naturalistic intensity series in paper (II) were analysed by the triple-extrapolation method (Juusola & de Polavieja 2003). The shot-noise responses in paper (III) were analysed using the method developed by Takalo et al. (Takalo et al. 2011), which works for any kind of input-output pair, providing enough data is available.
5 Results

This chapter summarizes the main results from original papers I, II and III.

5.1 Electrical properties (I)

Whole-cell voltage-clamped photoreceptors had high input resistances near the dark resting potential (1.6 ± 2.4 GΩ, min 200 MΩ, max = 10 GΩ, n = 32) and large whole-cell capacitances that ranged between 100 and 800 pF (Fig. 5). The capacitance varied considerably, suggesting variability in rhabdomere size, cell size or both.

Two types of voltage-gated potassium (K\textsubscript{v}) conductances were found in voltage-clamp experiments (Fig. 6A-C). The K\textsubscript{v} currents could be separated either by voltage protocols (Fig. 6D-F) or pharmacological agents. A slowly-activating, non-inactivating sustained type K\textsubscript{v} conductance (KDR) activated during depolarization and remained active during prolonged command voltage pulses. In most cells the sustained KDR conductance was accompanied by a transient inactivating A-type K\textsubscript{v} conductance (KA), which could be isolated using a voltage-clamp subtraction protocol (Fig. 6D-F). From the subtraction protocol data the voltage-dependence and kinetics of both activation and inactivation were analysed (Fig. 7) to be incorporated in a mathematical model of the photoreceptor (c.f. Materials and methods).
Fig. 6. The $K_v$ currents (transient KA and sustained KDR) were activated by depolarization, the relative amplitude of currents varied between photoreceptors. In A)-C) the voltage was clamped from -47 mV to +3 mV with 10 mV intervals after a -117 mV pre-pulse. A) Photoreceptor with large KA current B) Photoreceptor with both KA and KDR currents C) KDR current did not inactivate during a 10 s long voltage clamp. D-F) KDR and KA could be separated with a voltage-clamp subtraction protocol. D) Depolarizing pulses from -57 to +3 mV given after a hyperpolarizing -117 mV pre-pulse elicited both KDR and KA currents E) A positive pre-pulse inactivated the KA component, and subsequent depolarization activated only the KDR current. F) KA current could be isolated by subtraction of the currents from protocols in D) and E). The scale bar applies for panels D, E, and F. From I: Salmela et al. (2012)
Fig. 7. Voltage-dependence of activation and inactivation of the K\textsubscript{v} conductances determined from voltage-clamped photoreceptors. A) Steady state activation and inactivation properties of the K\textsubscript{v} currents (all data are mean ± SD). Black squares: KDR activation (n = 6); grey symbols: KA activation (circles, n = 5) and inactivation (triangles, n = 4). The curves are the corresponding Boltzmann fits (Eq. 2, see Table 1). B) Activation time constants of KDR (black squares, n = 8 to 14) and KA (gray circles, n = 5). KDR activation time constant was fitted with a bell-function (Eq. 3, see Table 1). C) Time constant of the KA inactivation (n = 3 to 7) and the bell function fit (Eq. 3). Inset: the inactivation recovery protocol used for voltages below -80 mV. From I: Salmela et al. (2012)

The variable whole-cell capacitance (Fig. 5) was associated with other electrical properties as cells with smaller capacitances had larger KA and smaller KDR conductances (see Fig. 5 in I). With prolonged light stimulation small-capacitance cells also depolarized less than cells with larger capacitances.

In addition to the K\textsubscript{v} conductances, a small-amplitude inward current activated by membrane hyperpolarization was found (Fig. 7 in paper I). The current resembled the CLC-2 type chloride current found in Drosophila photoreceptors (Ugarte et al. 2005).

Participation of KDR and KA conductances in shaping the voltage light responses was studied with a mathematical model of the photoreceptor. The model was based on the electrical parameters (Fig. 7), as well as the light-induced conductance determined from light-induced currents recorded under voltage clamp. The model suggested that the sustained KDR conductance activated during the simulated light responses and remained active for the duration of the light response. Modifications to KDR parameters in the model altered the light responses as well: decreasing the KDR maximum conductance led to larger amplitude and slower dynamics of responses, whereas increasing the conductance
resulted in smaller response amplitude and faster dynamics (Figs. 8, 9). Also, in case of the bump-size responses, decrease of the KDR would mean easier fusion of the bumps, leading to poorer temporal resolution (Fig. 9A). Interestingly the transient K_v conductance, KA, had no effect with the standard parameters, and modulation of maximum conductance or kinetics had no effect in either macroscopic (Fig. 8B) or bump responses (Fig. 9B) within the parameter range observed in our experiments. Thus the simulations showed that KA plays no significant role in the graded light responses in the photoreceptor soma, at least for the experimental conditions used here.

Fig. 8. Simulated light responses and the effect of varying K_v conductances. A) Light responses simulated with 10% (top trace), 100% (grey trace), 200% and 1000% (lowest traces) of the experimentally determined mean KDR maximum conductance demonstrate the effect of KDR on the light response amplitude and dynamics. B) Modifying the maximal KA conductance to 0%, 100%, 200% or 1000% relative to the standard simulation value of 60 nS had no effect on the simulated light response, which is why the traces overlap. (From Salmela et al. 2012)
Fig. 9. Simulated bump responses with modified KDR and KA maximum conductance. A) Changing the KDR maximum conductance to 10% (top trace), 100% (grey trace), 200% and 1000% (lowest traces) of the experimentally determined mean value altered the gain and temporal filtering of bump signals B) Modification of the maximum KA conductance to 0%, 100%, 200% or 1000% relative to the standard simulation value of 60 nS had no effect on the bump voltage waveform (all traces overlap).

5.2 Photoresponse dynamics (II)

Cockroach photoreceptors were previously found to differ in their adaptation kinetics and ability to maintain a depolarization for prolonged light stimulation (Heimonen et al. 2006). Here the contrast coding was studied in different functional types of photoreceptors by recording and analysing voltage and current responses to various types of light stimuli at different stages of light- and dark-adaptation.

Responses to step-like contrast changes given at specific light-adaptation background intensity were recorded from different functional photoreceptor types. In “adapting” cells both the steady-state depolarization and the maximum responses to contrast steps saturated at ca. 1,000 photons/s background intensity, after which increasing the intensity did not induce changes in the responses (Fig. 2 in II). “Hyper-adapting” cells completely failed to respond to contrast changes and their steady state depolarization was very close to the resting potential of dark-adapted cells, with single photon events occurring even at the brightest adapting background intensities.
Photoreceptor frequency responses measured in vivo with Gaussian white noise (GWN) light-stimuli demonstrated strong low-pass filtering features, with a -3 dB cut-off frequency at ca. 20 Hz. Both gain and phase remained relatively constant at all intensities over 1,000 photons/s showing very little adaptation-induced changes. Light-adaptation typically speeds up photoresponses, resulting in higher cut-off frequency and decreased phase lag, but in cockroach photoreceptors these effects were either small or altogether non-existent.

In “adapting” photoreceptors the coherence of GWN light responses improved slightly with increasing stimulus intensity, but was found to be generally poor. “Fast-adapting” or “hyper-adapting” photoreceptors had always low coherences regardless of the stimulus intensity. This is because of the light-adapted photoreceptors’ inability to maintain a depolarization during stimulation and the typical behaviour of “hyper-adapting” cells to adapt to a state reminiscent of dark-adaptation, where discrete bump responses occur even during bright light stimulus. Hence the poor coherence was likely to be a result of low SNR, caused by the photoreceptors inability to code the stimulus contrast into graded potentials. Coherences measured with naturalistic intensity series (NIS) stimuli were substantially higher than when analysed with GWN stimuli.

Information rates were estimated from the in vitro GWN SNR and in vivo NSI SNR using the linear Shannon estimator. Furthermore, short (2 s) NIS responses were analysed with the triple extrapolation method, which is a general information rate estimator that requires neither linearity nor Gaussian distribution of signals (Juusola & de Polavieja 2003). Information rates of NIS responses were much higher than with WN responses.

5.3 Shot noise mediated information (III)

Since phototransduction is limited to low transduction rates during night and in the cockroach even in bright light (II), it is important to know how much information shot noise initially contains, i.e. what is the physical limit of information transfer with low-intensity stimuli. Moreover, as photoreceptors add physiological noise on top of the physical noise, I wanted to study how a noisy phototransduction cascade further degrades the information content of an already noisy photon input. As it is not possible to simultaneously measure the occurrence of a photon emission from our light source and the photoreceptor’s response to
that specific photon, I resorted into modelling both the photon input and the photoresponse.

Since cockroach photoreceptors seem to transduce only ca. 1,000 photons/s, their information transfer is bound to be limited by the shot noise resulting from the low photon transduction rate. The shot noise information transfer rates and the average information carried by a single photon for GWN and 1/f contrast stimuli obtained by the Shannon and Kraskov-Takalo methods are shown in Figure 8.

![Graph showing shot noise information transfer rates for GWN and 1/f contrasts, analysed with both Shannon and Kraskov-Takalo information rate estimators.](image)

**Fig. 10. Shot noise information transfer.** A) Shot noise information rates for GWN and 1/f contrasts, analysed with both Shannon and Kraskov-Takalo information rate estimators. B) Information scaled to bits/photon for the data in A (inset: contrast and information rate estimator types).

Low intensity Gaussian white noise stimuli (GWN) had poor coherence and consequently low SNR values, resulting in very small information rates. Information rates obtained with 1/f stimuli were a little bit larger than with GWN; although at lowest intensities the rates with Shannon and Kraskov-Takalo methods were just about equal (Fig. 10A).

The shot noise information transfer rate limit is set by the stochastic properties of the photons and ultimately it is the best an ideal photoreceptor can do. However, biological photoreceptors are far from ideal due to various internal noise sources. Hence the effect of phototransduction noise was studied by simulating bumps with variable amplitude and timing, based on bump parameters obtained in whole-cell patch clamp experiments.

The effect of variable bump amplitude was negligible with both GWN and 1/f, analysed either by the Shannon or Kraskov-Takalo information rate estimator. Conversely, the variable bump latency had a significant effect for GWN stimuli analysed with Shannon (Fig. 11A), but only a small effect was found for GWN
using Kraskov-Takalo estimator (Fig. 11B) or for 1/f stimuli analysed with either of the rate estimators (Fig. 11C, D). Thus the information rates in the low-intensity regime are limited by the stochastic properties of shot noise alone and phototransduction noise is not large enough to have a measurable effect on the information rate.

![Graphs of information rates for different noise types](image)

**Fig. 11.** Shannon and Kraskov-Takalo information rates for light induced conductances containing various types of noise. A) GWN stimulus analysed with Shannon estimator B) GWN analysed with Takalo estimator C) 1/f with Shannon D) 1/f Kraskov-Takalo. Inset: noise type symbols.
6 Discussion

6.1 Implications of the study

Compared to previous studies on photoreceptors of nocturnal insects, some of the results were typical, while others were found out to be contrary to previously formulated hypotheses. Some functional properties of photoreceptors, such as ion channel composition of the non-transductive membrane, had not been studied previously in species that are nocturnal. Next I shall discuss the main findings in the light of the other studies carried out on vision of nocturnal insects.

6.1.1 Electrical properties of photoreceptors

The high input resistance and capacitance of cockroach photoreceptors were as could be expected for a nocturnal insect, providing the photoreceptors with large membrane gain (in the sense of mV/photons absorbed) and a slow membrane time constant, which together promote temporal pooling of the transduced signal. The large membrane gain enables generation of large single photon responses, or bumps, which can be several millivolts in amplitude. All these features tend to enhance bump-level signalling and temporal pooling, both of which are common strategies in night vision (Laughlin 1990, Warrant 2006).

The total cell capacitance (Fig. 5) reflects the photoreceptor’s membrane area, which in microvillar photoreceptors is substantially increased by the rhabdomere compared to a cylindrical approximation of the cell without any highly convoluted membrane, i.e. microvilli. Thus the large capacitances of cockroach photoreceptors result for the most part from large rhabdомeres commonly found in nocturnal insects with fused rhabдomes (Greiner 2006b, Greiner et al. 2004, Narendra et al. 2011, Somanathan et al. 2009). The wide capacitance distribution could result from different size groups being sampled in the experiments. In the Indian stick insect Carausus morosus the whole-cell capacitances vary substantially not only between different developmental stages, but also within the same age group (Frolov et al. 2012). Butler (1971) reported that out of the five green-sensitive rhabдomes in cockroach ommatidia, one was always small and one large, the rest of the rhabдomes varying in size according to the depth of the transverse cut along the ommatidium.
While the input resistance and capacitance of cockroach photoreceptors were very much what could be expected, surprisingly, the $K_v$ conductances were more akin to those previously described in diurnal or crepuscular insects. Earlier work on photoreceptor $K_v$ channels in flies (Anderson & Hardie 1996, Hardie 1991, Laughlin & Weckström 1993), locusts (Weckström 1994) and the Indian stick insect (Frolov et al. 2012) have associated the expressed channel types to the circadian activity of the species. In those insect species that are subject to low phototransduction rates (i.e. small number of photons/s), such as the nocturnal and crepuscular flies, or the diurnal stick insect nymph with highly insensitive photoreceptors, the dominant $K_v$ conductance type is transient. Conversely, diurnal flies and nocturnal stick insect adults with highly sensitive photoreceptors experience high phototransduction rates (larger number of photons/s) and require a sustained $K_v$ conductance to decrease the membrane gain in order to prevent saturation of the voltage responses.

In the cockroach, two $K_v$ conductances were found: a sustained type (KDR) and a transient type (KA). While KDR adjusts the membrane gain during light-induced depolarization, the voltage-dependence (Fig. 7) and the simulations (Figs. 8, 9) suggest that KA does not activate substantially during the graded light responses in the photoreceptor soma. However, cockroach photoreceptors have elongated axons where KA might have a function. In flies, photoreceptors with axons of different length - the long and short visual fibres ($lvf, svf$) - express a characteristic subset of $K_v$ conductances (Anderson & Hardie 1996). $Lvf$ axons travel through the lamina and synapse to medulla, whereas $svf$ axons synapse into lamina. The somata of $lvf$ cells are smaller those of $svf$ cells, which is why $lvf$ cells have larger input resistances and lower capacitances. The $svf$ cells in the blow fly Calliphora express a fast and a slow delayed rectifier, whereas in Drosophila the $svf$ cells express a slow and a fast delayed rectifier ($Shab, Shal$) and a fast inactivating A-type ($Shaker$) $K_v$ conductance. In both species, the slowest conductance is absent in the $lvf$ cells. The $svf$ photoreceptors in flies belong to the achromatic visual pathway that forms the input to the motion sensitive circuits in the brain, whereas the $lvf$ cells form the chromatic pathway responsible for colour vision. The motion sensitive pathway requires high temporal acuity, which is achieved by expressing a delayed rectifier conductance in the photoreceptors. In $lvf$ cells the temporal acuity is sacrificed for higher membrane gain required for transmitting the graded signal over the long $lvf$ axon by omitting the slower delayed rectifier component and expressing the transient
Kv conductance. Following this it can be noted that the transient KA conductance in cockroach photoreceptors was larger in cells with lower whole-cell capacitance, indicating that they are physically smaller, or at least their rhabdom is small. Since cockroach photoreceptors have long axons the transient KA conductance could serve a similar function as suggested for the transient conductance in fly lvf cells. The different sizes of the photoreceptor cells and the correlation of the rhabdom size to the connection pattern to lamina or medulla still remains to be investigated.

While light responses in the soma are graded, in vivo photoresponses recorded near the axon initiation with sharp intracellular electrodes contain a transient spike-like component (Weckström et al. 1993). The spikelet amplitude depends on the location of the recording electrode, as recordings done from the axon initiation show larger spikelets than recordings from the proximal soma. The spikelet has been suggested to be a remnant of a back-propagated action potential, originally generated somewhere along the axon. A full-blown action potential, or even the amplification provided by a sub-threshold action potential as in the honeybee drone (Vallet et al. 1992, Vallet & Coles 1993), could boost the conduction of small amplitude voltage responses in the long and thin axons. Photoreceptors have also been shown to have highly variable responses in terms of sensitivity and dynamics, and when several axons from different photoreceptors are pooled they can form a population coding system that is able to transmit more information than a group of identical photoreceptors (Heimonen et al. 2006). Transient Kv conductances in spiking cells can tune action potential generation and timing (Hille 2001), and a plausible role for KA conductance might then be in modifying the action potential initiation or propagation in the axons. However, since the axons are removed in the isolation process required for patch-clamp experiments, no spikes are seen in the whole-cell experiments (taking some credibility from the whole-cell term in this case). Clearly detailed investigation of the spikes and the signal coding in the axons in vivo is needed, although that be fairly challenging because of the small diameter (\(\sim 1 \mu m\)) of the axons.

Prolonged stimulation with bright intensity light sometimes causes cockroach photoreceptors to display a strong after-hyperpolarization (Fig. 2 in II). The hyperpolarization is reminiscent of the situation in blow fly photoreceptors, where the hyperpolarization is caused by Na/K pump activity that restores the ionic balances after influx of Na through the light-dependent channel opened during
light responses (Gerster et al. 1997, Jansonius 1990). The inward-rectifying conductance in cockroach photoreceptors, IR, activates with hyperpolarisation and should therefore decrease the otherwise enormous input resistance, which together with the pump current would cause massive change in the membrane potential, hyperpolarising the cell to a very negative membrane potential. The IR conductance would therefore be essential for limiting the pump-induced potential change to moderate values during the pump activity. However, the after-hyperpolarization is not always seen, and in e.g. intracellular recordings conducted with sharp microelectrodes the responses are more likely to contain a prolonged after-depolarization (a short segment of which can be seen in e.g. Figure 1 in II). The difference might be due to the different experimental conditions and in the different ionic balance in patch-clamp vis-à-vis intracellular recordings.

6.1.2 Photoresponse dynamics

Photoreceptors transform the energy of light reflected (or sometimes emitted) by objects into an electrical signal. The amount of light varies a lot with ambient illumination while the reflectance of objects stays about constant. Photoreceptors have thus evolved to code object contrast instead of absolute luminance. The large span of natural light intensities requires that photoreceptors adapt in order to encode the contrast into a limited membrane potential range. How do cockroach photoreceptors code contrast and what is the effect of light-adaptation on the coding?

Temporal dynamics of light responses in nocturnal insects are generally very slow. In Megalopta, when the photoreceptors are nearly dark-adapted and absorb 140 photons/s on average, the cut-off for the linear gain of the frequency response is at ca. 7 Hz and with light-adaptation in bright light (1.5 \times 10^6 photons/s) the cut-off increases to ca. 21 Hz (Frederiksen et al. 2008). In the cockroach the situation is different as light-adaptation does not significantly improve the bandwidth and the cut-off remains at ca. 20 Hz at all intensities. Contrary to other studied species, the light-adaptation in cockroach photoreceptors seems to halt at around 1000 photons/s and responses are transduced at a very low maximum photon rate regardless of the amount of light available. This suggests that the adaptation of cockroach photoreceptors to low-intensity light is so strong that the information
provided by brighter light will be disregarded by the visual system. What would be the benefit of having this kind of visual system?

The apparent constancy of the phase response in cockroach can be explained by the photoreceptors’ dead-time, defined as the time between stimulus onset and macroscopic voltage response. In cockroach the dead-time in both macroscopic voltage responses (Heimonen 1999) and single photon current responses (III) is ca. 16 ms. The effect of dead-time to the phase function is demonstrated in Figure 12, where simulated phase responses of 1st order low-pass filters containing 15 ms long dead-time are shown. The phase is dominated by the dead-time, \( \phi(f) = e^{-dt/T^2}\pi f \). Thus the possible changes in coding dynamics are not seen in the phase function, unless the dead-time is decreased.

![Figure 12](image_url)

**Fig. 12. The effect of dead-time on simulated phase responses.** Gain and phase functions of 1st order Butterworth low-pass filters containing a 15 ms time delay. The cut-off frequencies \( (f_c) \) were 10; 20 and 50 Hz. The phase responses are practically identical regardless of \( f_c \).

One possible explanation for the lack of light-adaptation effects in cockroach photoreceptors is energy saving. Photoreceptors need to maintain the ionic gradients, which are unbalanced by the influx of light-gated currents and the outflow of potassium during light responses. The ionic balance is restored primarily by a pump/exchanger that uses ATP as its energy source. The relation between photoreceptor performance and energy consumption has been studied in
flies by Niven and Laughlin (Niven et al. 2007), who showed that having faster photoreceptors that are able to code more information leads to increased energetic costs compared to having slower photoreceptors. The responses can be made faster by investing in the dynamics of phototransduction and the membrane impedance. Fast photoreceptors can thus be made with fast phototransduction coupled to a fast, i.e. leakier, membrane with lower resistance. Having a more energy-efficient (i.e. less leaky) membrane increases the impedance and limits the ion flux. Another hypothesis linking the photoreceptor performance to energy consumption is the relationship between the animal's visual ecology and the types of expressed \( K_v \) channels in the photoreceptors. In this sense the cockroach is a bit peculiar. Despite being a nocturnal insect with slow photoreceptor dynamics, it has a sustained \( K_v \) conductance proposed to fit the visual ecology of fast-flying diurnal insects with fast photoreceptors. Since the conductance does not inactivate, it will contribute a constant ion flux that needs to be pumped back into the cell by the Na-K ATPase, which requires energy. Then again, the limited phototransduction rate is energetically optimal because having fewer active transduction channels requires less work when balancing the concentrations.

One consequence of the finding that cockroach photoreceptors limit their transduction rate to maximum of ca. 1,000 photons per second is that shot noise is the most significant source of noise, even in daylight. The information transfer rates achievable by shot noise signals are very low, and as other nocturnal insects active in shot-noise intensities, cockroach too needs to apply spatial pooling of photoreceptor signals in the 2nd order cells in lamina and medulla to achieve at least moderate temporal and spatial resolution.

6.1.3 The properties of shot noise stimuli and responses

During naturally illuminated night the visual systems will sample trains of random photons, i.e. shot noise. Several animals – based on their behaviour – can see in such conditions, indicating that shot noise contains some significant information, albeit unreliable, that can be extracted by the visual system. Judging from the light-adaptation experiments in (II), it looks as if cockroach photoreceptors operate at low photon capture rates even in bright light, giving a major part of the transduced input properties of the shot noise. This was the motivation for studying shot noise limited information transfer rates and to
investigate how much worse phototransduction noise makes the situation compared to the shot noise alone (III).

The result here, that transduction noise (most importantly latency noise) does not lower the ultimate limit set by shot noise to any essential extent, is interesting, because it implies that photoreceptors can safely sacrifice precision for sensitivity without impairing their performance. Hence the poor performance of graded responses as shown in II and in Heimonen et al. (2006) could in principle indicate a “permanent” adaptation to shot noise input, where the phototransduction noise does not matter. Interestingly, the latency distribution and shape of bumps in Drosophila are almost identical to cockroach, while the bump amplitude is much smaller. Drosophila is mainly crepuscular and thus subjected to low-light ambient illumination in their natural habitat. The conclusions for shot-noise information processing can thus be appended to Drosophila as well, then, in the lower range of intensities where they are active.

The unsuitability of Shannon information theory to a shot-noise driven system results in a small positive bias in the information rate estimates at low-light intensities. This means that in the intensity regime where e.g. Megalopta genalis is reported to have functional vision, the information rates are actually even smaller than the Shannon estimates implicate. The absolute error, however, is small as the difference between the Shannon and Kraskov-Takalo rates for GWN shot noise for example at 10 photons/s was less than 1 bit/s. Nevertheless it becomes apparent that at light intensities encountered by nocturnal insects, the stimuli as well as the analyses should be chosen carefully in order to obtain realistic estimates of information transfer rates.

If GWN stimuli are not ideal for studying low-light vision, what kind of stimuli should be used then? Naturalistic contrast series stimuli have been shown to elicit larger responses, which can transmit more information (Faivre & Juusola 2008, Heimonen et al. 2012, Juusola & de Polavieja 2003), a finding which was confirmed also in II. These studies used stimuli taken from the van Hateren naturalistic contrast database (van Hateren 1997), which consists of data recorded by walking outdoors while wearing a light detector equipped helmet, and thus the data correspond to the spatiotemporal contrasts observed during walking. But how natural are these for an insect? Together the mode of locomotion and the spatial structure of the environment determine what kind of optic flow the animal encounters. A bee flying under a canopy sees an isotropic clutter of leaves and branches whereas a cockroach can climb surfaces and encounter all kinds of
structures and features on its’ foraging trips, including visually extended edges and borders. Visual systems have evolved to process certain type of optic flow and the stimulus should therefore match both the natural habitat and mode of locomotion for the studied species. The aspect of cockroach vision clearly calls for further investigations, with different types of visual stimulation. However, the higher order correlation that complex environment necessarily contain (Ruderman & Bialek 1994, Simoncelli & Olshausen 2001) are likely to be analysed at much deeper levels than in photoreceptors, and it is not at all clear, what substrates of this analysis would be needed from the photoreceptors.

Cockroaches tend to follow walls or wall-like structures whenever possible, with one antenna touching the wall ant the other antenna scanning the surroundings (Camhi & Johnson 1999). In this kind of behaviour the optic flow will become very asymmetric between the eyes. Does head movement affect the optic flow? Flies perform saccade-like movements during both free and tethered flight, but how do these affect the sampling of a visual scene? While naturalistic scenes are better known, cockroaches might behave differently in laboratory settings.

For the cockroach, mechanosensory input provided by cerci and antennae probably forms the primary information source, vision being a complementary sensory modality. For example, the role of vision during odour plume following is negligible (Willis et al. 2011). To what extent do cockroaches use their vision then? Best evidence for visually guided behaviour comes from studies, where cockroaches were shown to be able to exploit visual landmarks for navigation in a test arena (Brown & Strausfeld 2009, Mizunami et al. 1998). Besides landmark navigation, cockroaches use vision for antennal steering (Ye et al. 2003) and obstacle negotiation (Harley et al. 2009). Unfortunately none of these studies tested the visual capability in low-light conditions.

6.2 Reliability and validity of the study

The results of this thesis are based on combination of experiments and simulations, both of which are prone to errors. The most important methodological issues and limitations are discussed in this chapter.
6.2.1 Experiments

Electrophysiological methods allow us to study the photoreceptors in action and see what kind of mechanisms lay behind the generated light responses. However, the recordings are prone to errors, which are mostly caused by the properties of electrodes and the large whole-cell capacitance of the photoreceptors. Patch electrode resistances normally range from less than MΩ to tens of MΩ. In whole-cell experiments, the resistance can be partially compensated, but the instability of the compensation circuitry limits the compensation to 90% at max, leaving a small residual series resistance. The seriousness of the series resistance error depends on the amplitude of the currents that the electrode has to pass. Cockroach photoreceptors are relatively large cells, capable of generating large currents. For example, the K$_v$ currents studied in this thesis can be several nA in amplitude. The large current flowing through the electrode generates a voltage drop across the series resistance, for example 5 nA current passing through a 5 MΩ resistance creates an error of 25 mV. As a result the cell is clamped in other voltage that was originally planned unless the voltage error is accounted for. Figure 13 shows an example of an activation curve for the cockroach KDR conductance, with and without voltage correction.

![Fig. 13. Effect of uncorrected series resistance on conductances. The Rs corrected conductance curve was simulated with Eq. 2 using $V_{50} = -31$ mV; slope = 12 mV and $G_{KDR} = 78$ nS. KDR current was then calculated from conductances as: $I_{KDR} = g_{KDR}(V)(V - E_K) = g_{KDR}(V)(V +68mV)$. From the currents, conductances with Rs error were finally calculated as $g_{Rs}(nS) = I/(V - E_K + I*Rs)$, with 5MΩ Rs. The uncorrected curve has larger $G_{KDR}$ and $V_{50}$.](image-url)
The problems related to large currents and the series resistance can be partially corrected by estimating the steady-state voltage error, but voltages during current transients are more difficult to correct and thus less reliable than steady-state or slow current responses. Since cockroach photoreceptors have relatively large capacitance caused mostly by the large rhabdomeres, the capacitive transients resulting from the whole-cell capacitance and the series resistance are large and the time constant of the decaying phase is slow. Hence for KA the fast activation kinetics could not be accurately characterized because the activation overlapped with the capacitive transient.

The input resistances recorded in whole-cell patch clamp were higher than previously reported with sharp intracellular electrodes (Heimonen et al. 2006). This is probably caused by the absence of an electrode-induced leak during whole-cell recordings, but which is known to occur during impalement of the cell membrane with a sharp microelectrode typically used for in vivo recordings (Georgiou et al. 1987, Li et al. 2004). The input resistance values obtained from isolated ommatidia may also be affected by changes in the cell induced by the ommatidium isolation process, e.g. due to detachment of the photoreceptor axon.

The poor SNR of cockroach photoreceptors (as in II and in Heimonen et al. 2006) is likely to result from both the properties of the photoreceptor and the light stimulus. The bandwidth of the Gaussian white noise stimulus, 500-600 Hz, was determined by the sampling frequency light stimulus control signal. Since the signalling bandwidth of cockroach photoreceptors is very narrow (cut-off frequency of frequency responses was ca. 20 Hz), most of the light stimulus power thus lies outside the receptor bandwidth. At the moment there is no clear agreement on how wide the stimulus band should be in relation to the response bandwidth. SNR in other studied species have been assessed with narrower bandwidth stimuli compared to their responses and the higher SNR values obtained in the case of those species might reflect the better match between the response and stimulus bandwidths. On the other hand, using similar GWN stimuli in different species allows comparison of their performance to that specific GWN stimulus, but this naturally leads to the species adapted for different signal bandwidths being tested in conditions where they are not supposed to be working.
6.2.2 Modelling

“Essentially, all models are wrong, but some are useful” –George E. P. Box

Perhaps the most useful feature of modelling is the full control of parameters, allowing investigation of individual and interacting mechanisms to the overall functioning of a complex system. Modification of parameters in experiments is difficult, since e.g. pharmacological agents are often unspecific and using gene mutations may induce compensatory effects, such as the increased leak conductance in response to deletion of a $K_v$ conductance in *Drosophila* photoreceptors (Niven *et al.* 2003a, Vähäsöyrinki *et al.* 2006). However, genetics tools are improving and genomes are being published constantly, though, and we may soon have access to valuable equipment for studying gene function in insects other than *Drosophila*. But meanwhile we have to settle with the still fairly fruitful combination of electrophysiology and modelling.

The models in this thesis were based on the parameters obtained from experiments. Moreover, the models used mean parameter values obtained from several photoreceptors. Since the cockroach photoreceptors have been reported to show large variability in almost every parameter (Heimonen *et al.* 2006), using a fixed set of mean values might not represent the functional properties of any single cell selected from a larger population (Golowasch *et al.* 2002). As a partial remedy for this, the models were generally tested by varying parameters within the ranges found in experiments.

The estimate of macroscopic light conductance was based on recorded light-induced currents (LIC). In the cockroach, LIC amplitude can be several nA, making accurate voltage-clamping difficult (c.f. previous chapter on the reliability of experiments). This may cause errors in the estimated light-induced conductance, as the driving force (difference between the actual holding potential and the reversal potential of the light-induced current) might not stay constant as assumed in the calculations. The LIC also varies substantially between photoreceptors, and it is likely to be the prime cause behind the variability of photoresponses (II). The HH-like model of the $K_v$ channels was tested with several LIC waveforms, of which a typical response was taken as the model input. However, the light response variability might be connected to other electrical properties, such as $K_v$ channel expression or whole-cell capacitance, both of which were found to correlate with the light response amplitude (I). Thus some
compromises had to be made in order to capture some essential features of the photoreceptor function, leading to the use of (mainly) the mean and thus “typical” values of the parameters.

Both photoreceptor models (I, III) were isopotential, meaning that membrane voltage was uniform across the membrane area. In reality photoreceptors are far from isopotential, as the soma contains a microvillar and non-microvillar membrane with different ion channel localization (Hardie 1991, Rogero et al. 1997). Furthermore, cockroach photoreceptors have very long axons bundled within myelin sheaths, with several axons traveling along each other. The axons generate spikes, which may result in cross-talk between the densely packed axons inside myelin insulation. At the moment, however, the available data do not allow accurate modelling of axonal signalling or the possible effects of axonal coupling. Therefore these complications were ignored in the modelling. However, models with similar structure can be used in future work, when more experimental data on the axon signalling will be available.

6.3 Suggestions for further research

The “big picture” regarding the voltage-dependent conductances in nocturnal photoreceptors is still relatively uncertain and thus requires further studies. Since the whole-cell patch clamp method has been proven to work for other species besides Drosophila (Frolov et al. 2012), the link between the visual ecology and the voltage-gated channels will most likely be clarified in the future with extensive comparative work across different types of photoreceptors and across different taxa of insects.

6.3.1 Does KA have a function outside soma?

The failure of KA activation during graded potential responses in the soma leaves open the possibility for a physiological function in the photoreceptor axon. A-type Kv channels regulate various aspects of action potential signalling in spiking neurons (Hille 2001, Wicher et al. 2001). The spiking photoreceptors in cockroach compound eyes (Heimonen et al. 2006, Weckström et al. 1993) and Megalopta ocelli (Berry et al. 2011) suggest that in addition to graded potentials nocturnal insects may use spike-like signalling in their photoresponses, in which case an A-type Kv conductance such as the cockroach KA could be of use.
Whether the spikes are related to a boosting of graded signals akin to honeybee drone photoreceptors (Vallet et al. 1992) or contribute to a population code as hypothesized by Heimonen et al. (2006) – or both – remains to be shown by recordings from photoreceptor axons and 2nd order cells in the lamina or medulla.

6.3.2 What is the scale of spatiotemporal pooling required for making sense of shot noise responses?

Electrophysiological characterization of the Lamina monopolar cells is also essential for testing the theoretical predictions about spatiotemporal filtering in nocturnal insects (Klaus & Warrant 2009, Theobald et al. 2006), not only in the cockroach but in other species as well. As any single photoreceptor is unlikely to convey enough information to guide behaviour, the effect of spatial pooling of shot noise induced photoresponses should be tested. The testing is possible with combined modelling of shot noise processing by photoreceptors (presented in this thesis) and a model of the spatial pooling based on the reported anatomical findings of axon bundling and 2nd order cell dendrites. Even better, recordings from the 2nd order cells would give the spatial receptive field and a quantitative assessment of the first two stages of shot noise response propagation within the optic lobe.

6.3.3 Better information theoretical tools for analysing vision in low light?

Better tools for analysing the information transfer by low-intensity stimuli are required. Although the model allowed estimation the information transmitted by shot noise in cockroach photoreceptors, the experimental data contained components that interfered with the analyses. In our experiments, excess noise unrelated to signalling could be misread as information by the analyses, and should therefore be taken into account somehow. Time-domain averaging of responses does not work well with low-intensity stimuli because averaging removes the signal-carrying shot noise component. Thus, some other methods are needed.
6.3.4 Naturalistic stimuli for the cockroach?

The importance of the temporal properties of the stimulus became apparent in the dynamics (II) and shot noise (III) papers. The 1/f stimuli were not natural for cockroach though, but either taken from a database of naturalistic contrasts sampled by a light detector worn by walking human (van Hateren 1997) or produced with a random number generator algorithm (III). What would be the naturalistic stimulus for a cockroach? Cockroaches are semi-urban and may encounter various spatial structures while moving about. Since cockroaches move primarily on terrain following walls and other structures, the optic flow collected by the compound eyes is most likely to be very different from that of a walking man or a flying insect. The relevance of naturalistic stimulus to animal behaviour has been acknowledged, however, and hopefully in the future we will know more about the world as observed through the eyes of terrestrial insects.

6.3.5 Behavioural experiments at shot noise intensities

Even though the information processing capabilities of single photoreceptors can be estimated with various methods such as used in this work, the performance of night vision needs to be ultimately tested in a behavioural assay in order to show that cockroaches can actually both collect and use the information provided by shot noise input. Combined behavioural and electrophysiological studies of visual performance in low light conditions have been published previously from e.g. the housefly Musca domestica (Dubs et al. 1981), but comparable studies in nocturnal species are needed before photoreceptor performance can be linked to behaviourally relevant light intensities.
7 Conclusions

1) The high input resistance and whole-cell capacitance of cockroach photoreceptors were typical for a nocturnal insect, but the two $K_v$ conductances were unlike the conductances previously associated with a nocturnal life-style. The functional role of the transient $K_v$ conductance remains unknown, since the voltage dependence of the activation kinetics prevents it from activating during physiological light response voltages.

2) Photoresponses were slow and failed to speed up with adaptation to brighter light backgrounds. Phototransduction rates were limited to a maximum of ca. 1,000 transduced photons per second even in bright backgrounds, indicating that the photoreceptors are permanently adapted to low-light conditions. Due to better match with the photoreceptor’s signalling bandwidth, information transfer rates with 1/f contrast noise were substantially higher than with Gaussian white noise.

3) The information content of low-intensity contrast stimuli is dominated by shot noise properties of the random photons. Since cockroach photoreceptors transduce a maximum of 1,000 photons per second, they are always limited by the random properties of photons and the internal noise generated by the biophysical processes within the photoreceptor itself is of less importance. The random latency of single photon responses lowered the information transfer rate in the photoreceptor whereas the random amplitude of single photon responses did not.
References


Immonen E-V (2013) Visual transduction in cockroach photoreceptors is mediated by primarily by TRPL channels. Personal communication.


Original articles

The original articles have been omitted from the electronic version of the thesis.