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# TOWARDS NATURAL INSECT VISION RESEARCH

JOUNI TAKALO

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# TOWARDS NATURAL INSECT VISION RESEARCH

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**Abstract**

Visual world is naturally correlated both spatially and temporally. The correlations are used in vision to enhance performance of neurons. For gaining maximal neural performance of the visual neurons, the experiments, from stimulus to the analysis, should be designed to take advantage of the correlations. In this thesis methods for generating and analyzing natural stimuli were examined by using computations and algorithms.

For analyzing responses to natural stimuli in visual neurons, a method with only a few assumptions was developed for estimating information rate in long responses. The novel method gave a good agreement with Shannon information rate with linear system and Gaussian input but was able to handle also nonlinear processing and non-Gaussian data.

Secondly, a computer controlled 3D virtual environment with a spherical screen was developed, with a large visual field. The image of the world was projected to the screen with a DLP projector, giving good enough temporal performance for insect vision research. A track-ball was used in closed loop experiments.

Thirdly, properties of single photon (“bump”) information transfer at various light levels were investigated in cockroach photoreceptor with a coarse computational model. At dim light ( $< 10$  ph/s), where single bump responses were visible, shot noise was dominant. At higher light levels latency distribution of the bump decreased the information rate, but amplitude distribution of bump did not have an effect.

Fourthly, the contribution of  $K^+$  channels to information rate and energy consumption was investigated by creating a database of computation models with varying channel compositions. The information rate has a maximum as a function of mean conductance, which was a sum of the average  $K^+$  conductance and the leak conductance. This maximum was fine-tuned by the  $K^+$  channel composition,

which had high so-called novel contribution and relatively low amount of other conductances.

Keyword: insect, vision, Hodgkin-Huxley model, natural stimulus, information rate, virtual environment

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Oulu, October 2013

Jouni Takalo

## **Abbreviations**

ATP Adenosine triphosphate  
DLP Digital Light Processing  
EEG Electroencephalography  
fMRI Functional magnetic resonance imaging  
LED Light emitting diode  
LIC light induced conductance  
ph Photon  
RMS Root mean square

## List of original articles

- I. Takalo J, Ignatova I, Weckström M, Vähäsöyrinki M. A Novel Estimator for the Rate of Information Transfer by Continuous Signals. Plos ONE. April 2011;6(4):1-8.
- II. Takalo J, Piironen A, Honkanen A, Lempeä M, Aikio M, Tuukkanen T, Vähäsöyrinki M. A fast and flexible panoramic virtual reality system for behavioural and electrophysiological experiments. Scientific reports 2 (2012).
- III. Salmela I, Takalo J, Immonen E-V, Krause S, Krause Y, Weckström M. What do photoreceptors see in shot noise? (submitted)
- IV. Takalo J, Ignatova I, Niven JE, Weckström M, Vähäsöyrinki M. Are fruitfly Photoreceptors operating with optimum K<sup>+</sup> channel composition? (submitted)

My contribution to these articles or manuscript is generally algorithmical and computational. In addition, I have been mainly responsible for drafting the first version of the papers I and IV and participated in writing of the paper II and III. I have not done the experiments presented in these papers, but done the development and testing of the method in paper I, had main responsibility in programming of the virtual environment and technical measurements in paper II, analyzed the data in paper III, and in paper IV my responsibility was to simulate the database and I analyze the results. I have participated to writing all papers in sections which are relevant to my work.



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# 1 Introduction

For many animals, including humans, the visual world is important for their behaviour, which makes vision a good target for neuro-scientific studies. Especially insects have many stereotypical behaviours based on various visual stimuli (Paulk *et al.* 2013, Warrant 2008, Zeil 2012). Second advantage in visual studies is the relatively easy control of the light stimuli, because of the good knowledge of the physics of light generation.

Sensory neurons for visual stimuli are photoreceptors, which transform light to membrane voltage (Hardie & Postma 2008, Song *et al.* 2009). The process can be divided to two steps: the phototransduction and the processes in the photo-insensitive membrane. The phototransduction amplifies single photon signals in several steps in a biochemical cascade. The cascade processes lead to opening of ion channels, giving the initial current for the photo-insensitive membrane which transforms the current to the voltage. It consists of different type ion channels and capacitance of membrane which together make membrane filter (Hardie 1991, Weckström *et al.* 1991).

Because transfer of light information in photoreceptors is a complex process, the many studies make assumptions, which simplify the situation. But simultaneously the natural functionality of the photoreceptor is not completely assessed in the studies. Following simplifications are typical in the studies:

- Stimulus is set to be Gaussian white noise modulated light or current, which linearizes the response (Marmarelis & Marmarelis 1978, Spekreijse & Oosting 1970). But the Gaussian white noise does not resemble at all the natural visual environment with typically non-gaussian distribution of light intensities with 1/f-like power spectrum (Ruderman 1994, Van Hateren & Van Der Schaaf 1996). Therefore, the natural operation range of photoreceptors could not be analyzed with Gaussian white stimulus, because responses are limited to an artificially small voltage interval. This means also that operational ranges of voltage activated  $K^+$  channels are smaller than in the nature. Performance of photoreceptors is analyzed with a information theoretical approach using Shannon information capacity (Borst & Theunissen 1999, Shannon 1949, Shannon 1948), which assumes Gaussian distribution of the stimulus and the output (here photoreceptor voltage signals). Similarly as previously, the Shannon information capacity does not

give a good estimate of natural range performance. In the worst case, like the cockroach, a Gaussian stimulus generates only a very small response (Heimonen *et al.* 2012).

- Visual fields of insects are typically large. They see from front to almost back and from almost down to straight up, forming a visual field of 250-300 degrees. Many higher order neurons may hypothetically use large spatial pooling to increase their signal amplitude. The full pooling cannot be achieved with stimulus devices with small coverage of the visual area and which leads again to an underperformance of neurons if they do large spatial pooling.
- Photoreceptors absorb light quanta discretely, instead of a continuous stream of an input signal. This phenomenon becomes important when insects live in dim conditions, where quantum absorptions are separate enough to be visible in the response. The quantum effect is often neglected by looking only at the continuous responses in bright light. However, many behavioral studies suggest that responses to the quantal responses lead to visible change of the behavior of the insect (Warrant *et al.* 2004, Warrant 2008).

The general aim of this thesis is to make it possible to use complex stimuli and the appropriate analyses for visual experiments, which both, if successful, will enable us to do experiments under more natural visual conditions. The complex stimulation can be generated by computer controlled virtual environment (paper II), which realizes a fast stimulus and also large visual fields for insect studies. The complex analyses are done by the direct method for estimating information rate (paper I), so that it is possible to analyze the information rate of any continuous neural signal. The method described in paper I is then used in two simulation studies which were not possible with traditional methods. Paper III describes the information rate at dim light conditions in photoreceptors, where the response consists of a trace of individual bumps. In paper IV the effect of voltage-gated  $K^+$  channels to the information rate are analyzed.

## 2 Review of the literature

### 2.1 Insect vision

Information about the visual world is one of the main sources of sensory input for many insects, especially for flying insects. This can be seen just by looking at the size of eyes compared to body size of the animal. The biggest eyes in insects are compound eyes, which form two large curved surfaces on each side of the head. The exact form of the surface depends on species, but is normally roughly spherical. Therefore, insects have a large visual field. The surface of one optical type of eyes with pigment-separated small eyelets (the ommatidia), the apposition eye, is divided to ommatidia, whose spacing defines the spatial resolution of the compound eye. Each ommatidium has its own lens and, behind the lens, typically eight photoreceptors, which are sensory neurons for gathering visual information. The photoreceptors send the visual information to the higher order neurons by chemical synapses and eventually guide the visual behaviour of the insect.

Photoreceptors convert light to membrane voltage (Hardie & Postma 2008, Hardie & Raghu 2001). The first step is the absorption of light by rhodopsin. Because the absorption is a quantum phenomenon, each rhodopsin absorbs – at a time – only one light quantum, the energy of which matches the corresponding energy levels of the rhodopsin molecule. Insects have several types of rhodopsin, which together form the base of the colour vision. The most typical rhodopsins in insects have peak absorptions at green or UV light, but the absorption spectra cover commonly large parts of the visible light spectrum.

The absorption of the light quanta in microvilli starts a biochemical cascade, which amplifies single photon absorptions to thousands of molecular changes in the cascade (Song *et al.* 2009). Eventually the biochemical cascade opens TRP-type ion channels by chemical or possibly mechanical mechanisms in the membrane (Hardie & Franze 2012). The TRP-type ion channels pass current, which depolarizes the voltage over the cell membrane. Opposing this, the voltage activated  $K^+$  channels hyperpolarize the membrane, providing dynamic gain control for the membrane (Sinz & Bethge 2013, Van Hateren & Snippe 2001).

## 2.2 Visual field stimulus devices

Because the field of vision of an insect is typically larger than a half-sphere, the visual stimulus devices need to cover an angular area significantly larger than that, which is not possible with a normal flat screen, although it has also been used as stimulus device (Fry *et al.* 2004). A second complication for designing virtual environment stimuli for insects is the fast vision of many flying insects such as flies. For example, the corner frequency of the blowfly is over 100 Hz in the light-adapted state (Tatler *et al.* 2000).

The first versions of very large stimulus devices have been mechanical, such as a spinning drum, which has been used to study motion sensitive behaviour (Götz 1964, Strauss *et al.* 1997). These devices are easy to build, but they normally are suited for just one specific stimulus type. Nowadays the drum may be replaced with e.g a cylinder of LEDs (Kimmerle *et al.* 2000, Strauss *et al.* 1997). For more variable stimuli a fully computer-controlled environment is necessary. It allows a feedback loop between movement of the animal and the stimulus. The environment can then move according to the animal's movement, with the animal itself staying in place, which is important in neuron recordings. The visual stimulus may be based on LEDs (Lindemann *et al.* 2003), CRT screens (Fry *et al.* 2004) or projectors (Fry *et al.* 2008, Gray *et al.* 2002, Guest & Gray 2006). The shapes of these vary from flat screens to more than a half sphere. Both the screen and the projector are originally designed for human vision, which is normally much slower than the vision of insects. Therefore, only the fastest screens and projectors are suitable. Similarly, standard computer graphic engines are designed for human vision, which leads to a new kind of computational problems, where the time accuracy of the desktop computers, which is 1 ms, is not accurate enough for insect vision, due to the need for speed in fractions of the millisecond.

### 2.3 Photoreceptors at dim conditions

Light is constructed from quantum events, photons, whose energies are defined by the wavelengths of the light. In dim light the density of photons is sparse, which causes the quantum events - arriving at photoreceptors with different timing. This phenomenon is referred as the shot noise. Due to the high gain of the photoreceptors the photons may form a measurable response, if the gain of the biochemical cascade is large enough. The single photon signal is generally called a quantum bump and it may be measured either in the current or the voltage of the photoreceptor (Dubs *et al.* 1981, Howard 1983, Lillywhite & Laughlin 1979). Generally, nocturnal species have larger bumps than diurnal ones, but the exact shape of the bump is species dependent.

Photons arrivals follow the Poisson law, which states that observing  $k$  photons during an interval of  $t$  follows a Poisson distribution with mean photon rate  $r$  (Bialek 2012):

$$P[N(t) = k] = \frac{e^{-rt}(rt)^k}{k!} \quad (1)$$

This phenomenon can be recorded from photoreceptors with dim continuous light by measuring the bump rate (Dubs *et al.* 1981). However, it is still relevant in brighter light intensities, where bumps fuse to a summed response (van Steveninck & Laughlin 1996). The Poisson law sets a fundamental limit to information transfer of light, which is restrained by the Poisson probability distribution (Heimonen *et al.* 2012, Niven *et al.* 2007).

### 2.4 Simulating electrical membrane of photoreceptors

Cell membrane is modelled as an electrical equivalent circuit (Figure 1), where the lipid membrane corresponds to the membrane capacitor ( $C_m$ ) and where ion channels are modelled as conductors ( $g_x$ );  $x$  is the index of the channel,  $x=1,2,3,\dots$ . Voltage ( $V$ ) over the cell membrane is a function of the total current

(sum of  $I_x$ ) flowing through ion channels, which charges the membrane capacitance (Hodgkin & Huxley 1952):

$$\frac{dV}{dt} = -\frac{1}{C_m} \sum_x I_x + \frac{I_s}{C_m} \quad (2)$$

where  $I_s$  is the injected stimulus current and where the current of the channel is dependent on the driving force, the difference between the membrane voltage and reversal potential of the current through the channel ( $E_x$ ):

$$I_x = g_x \gamma_x (V - E_x) \quad (3)$$

where  $g_x \gamma_x$  is the conductance for the type-x channels. This consists of the maximal conductance  $g_x$  and the open probability part ( $\gamma_x$ ), which varies between channel types. The simplest one is the passive channel, when open probability is always 1. For voltage activating channels  $\gamma_x$  is a product of one or more gating probabilities ( $n_x$ ), which follow the differential equation of a two state system:

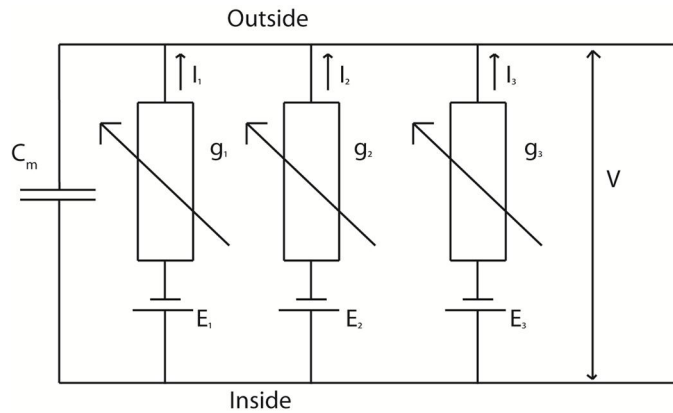
$$\frac{dn_x}{dt} = \frac{n_x^\infty(V) - n_x}{\tau_x(V)} \quad (4)$$

where  $\tau_x(V)$  is the time constant of gating, which has several different forms, depending on the gating of the ion channel. The steady-state equation of gating  $n_x^\infty(V)$  follows the Boltzmann equation:

$$n_x^\infty(V) = \frac{1}{1 - e^{\frac{V - V50_x}{dx_x}}} \quad (5)$$

where  $V50_x$  is the half activation of the channel and  $dx_x$  is the slope constant, both determined experimentally.



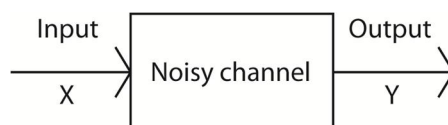


**Fig. 1. Electrical Circuit for the cell membrane, which consists of a capacitor and three different ion channels.**

## **2.5 Applying information theory to biological systems**

### **2.5.1 General definitions of information theory**

Information theory is used for analysing information flow through a communication channel by Shannon in his seminal article from 1948 where basic definitions and rules were made for information theory. In the information theory an input ( $X$ ) is transmitted to an output ( $Y$ ) through a noisy channel where input, output and noise are described as random distributions, which tell probabilities of the occurring channel states. In communication theory it is important to know how much information can be passed through the channel.



**Fig. 2. Schematic of information transfer through a channel**

The most basic concept of information theory is entropy, which tells what is the spread of a random distribution regardless of the shape of the distribution (Cover

& Thomas 2006, Shannon 1948). Here the entropy  $H(X)$  is defined as a function of a continuous random variable  $X \sim p(x)$ :

$$H(X) = - \int_x p(x) \log p(x) dx \quad (6)$$

where the integral is taken over non-zero values of  $p(x)$ . The base of the logarithm is most commonly 2, so that the unit of entropy is bit. There is also an entropy definition for discrete random variables, but it is not shown here. Similarly, the joint entropy  $H(X; Y)$  for the joint probability of  $X, Y \sim p(x, y)$  is:

$$H(X; Y) = - \int_{x,y} p(x, y) \log p(x, y) dx dy \quad (7)$$

The conditional entropy  $H(Y|X)$  is defined as the entropy of the conditional distribution between two random variables:

$$H(Y|X) = - \int_{x,y} p(x, y) \log p(y|x) dx dy \quad (8)$$

A stochastic process consists of a sequence of random variables:  $\bar{X} = \{X_k | k = 1, 2, 3, \dots\}$ . The stochastic process is assumed to be stationary, independent of time shift  $t$ :  $p(x_1, x_2, x_3, \dots) = p(x_{1+t}, x_{2+t}, x_{3+t}, \dots)$ , where  $t \in \mathbf{Z}$ . For the stochastic process the entropy rate ( $h(\bar{X})$ ) is comparable for entropy of the random distribution:

$$h(\bar{X}) = \lim_{n \rightarrow \infty} H(X_n | X_{n-1}, X_{n-2}, \dots, X_1) = \lim_{n \rightarrow \infty} \frac{H(X_1, X_2, \dots, X_n)}{n} \quad (9)$$

when the stochastic process is stationary.

The mutual information ( $I(X; Y)$ ) describes how much information two random distributions  $X$  and  $Y$  share (Cover & Thomas 2006, Shannon 1948):

$$I(X; Y) = \int_{x,y} p(x, y) \log \frac{p(x, y)}{p(x)p(y)} dx dy = H(X; Y) - H(X) - H(Y) \quad (10)$$

Note that if X and Y are independent then  $p(x, y) = p(x)p(y)$  and in this case the mutual information is zero. Similarly, for two stochastic processes the information rate is a similar measure as the mutual information (Khinchin *et al.* 1957):

$$R(\bar{X}; \bar{Y}) = h(\bar{X}; \bar{Y}) - h(\bar{X}) - h(\bar{Y}) \quad (11)$$

Shannon showed that there is a maximum information rate, generally called the channel capacity C, which can be achieved without error (Cover & Thomas 2006, Shannon 1949):

$$C = \max_{p(x): E[p(x)]^2 \leq P} R(X; Y) \quad (12)$$

Normally,  $p(x)$  has one or more constraints, because if  $p(x)$  is infinitely large, the channel capacity is infinite. In this case the most common restraint for the continuous channel is the following: the average power needs to be smaller or equal than P. Then the channel capacity can be found to be, in the case of the added Gaussian noise with power N:

$$C = \int_0^\infty \log(1 + SNR(f)) df \quad (13)$$

where the SNR is the signal-to-noise ratio ( $SNR(f) = P(f)/N(f)$ ) for every frequency and for multiple different bandwidths when P(f) is the signal power and N(f) is the noise power, all now expressed in function of signal frequency.

### **2.5.2 Using information theory in neuroscience**

Information theory has been used successfully in neuroscience for various applications. Most commonly information theory is used as a measurement of correlation of both graded and spiking neurons and from single neurons to populations of neurons (Borst & Theunissen 1999, Juusola & de Polavieja 2003, Juusola *et al.* 2007, Schneidman *et al.* 2003, Spavieri *et al.* 2010, Strong *et al.*

1998). This has been achieved by using different variations of mutual information to estimate the correlations in the activity of neurons. The strength of this method compared to classical calculations of correlation is that the mutual information uses probability distributions instead of chosen correlation functions that could be incorrect for a particular system.

Analysis of the neurons is dependent on the signals that the neurons generate. Spiking neurons have been analysed in two ways, depending on interpretation of neural coding: timing or rate code (French et al. 2003, Haag & Borst 1997, Strong et al. 1998). With the time code spikes are transformed to zeros and ones, for no spike and spike present in a time interval, respectively. The zero-and-one-code forms a base for discrete distributions. In the rate code spiking frequency is calculated with a moving window and the frequency forms a continuous signal that is possible to analyse with information theory. Information contents of graded neurons, such as photoreceptors, have been estimated similarly as the spiking neurons with the rate code assumption.

The most commonly used information theoretical method for continuous neural signals is to estimate information capacity using formula 13 (Borst & Theunissen 1999, Van Hateren & Snippe 2001). There are three ways for obtaining the estimate: an upper bound, a lower bound, and a direct method. In the upper bound method the mean response is calculated by averaging several responses and the noise is estimated as the difference between actual response and the averaged response. The ratio between the average response and the noise is the SNR, and the information capacity can be calculated from formula 13. The lower bound expects linear coding, whose fitness is determined by the coherence (see methods for more details). The direct method gives the information rate without the Gaussian distribution assumption by estimating probability distributions directly from repeated measurements, whose mean response is determined. This can be done both for binary neurons (Strong et al. 1998) and for continuous signals (Juusola & de Polavieja 2003). The entropy estimates have been used also to measure the spread of signals, although they are less common in the literature. However, for the most part the entropy has been a side result in calculating the SNR of the neural channel for average signal and noise (French et al. 2003, French & Pfeiffer 2011, Juusola & de Polavieja 2003, Strong et al. 1998).

Although information theory is a powerful tool to analyse information transfer reliably, it needs a large amount of data due to the required estimation of distributions (Juusola & de Polavieja 2003, Strong *et al.* 1998). Especially, this problem arises in information rate estimates where the distributions have multiple dimensions, which each worsen the accuracy of the estimate. This problem is called dimensional explosion in the literature (Nemenman *et al.* 2004, Victor 2002). Therefore, many methods to restrict or simplify the estimations have been developed.

Information theory provides a tool to analyse pairwise correlations without need of knowledge of the types of correlations. This is useful in many biological measurements, where correlations are complex, such as the correlation between EEG and fMRI (Magri *et al.* 2012). In the literature this analysis appears with many names, but the technique is the same, the mutual information is estimated between two time series with a varying lag. This method corresponds to the cross-correlation estimation in the linear case, but without restrictions of linear analysis. The maximum of the mutual information gives the average latency between the two time series.

### **2.5.3 Photoreceptor information rate**

Information rates of photoreceptors of various insects have been studied for quite some time due their easy access. Traditionally, investigators have used Gaussian white noise as the input (the light stimulus) and information has been estimated by using various forms of Shannon information capacity (formula 13) (Borst & Theunissen 1999, Juusola & Hardie 2001, Niven *et al.* 2007, Van Hateren & Snippe 2001). Most commonly the average light contrast, measured as standard deviation of light divided by mean of the light in the stimulation sequence, is fixed and the mean intensity of light is varied. Often the contrast is fixed to ~0.3, because it is the maximal contrast in a fixed range, which is possible to generate, because a higher contrast gives higher information rates (Juusola *et al.* 1994). All studied insect species show an increasing information rate as a function of the mean intensity. Maximal information rates obtained with this method have been near 1000 bits/s for fast day active flies in light-adaptation. Faster vision generates higher information rates. However, in the other situation this method doesn't function at all. Heimonen *et al.* (2012) showed with the cockroach photoreceptors that they did not generate proper responses to this kind of stimuli,

although a so-called naturalistic stimulation (with  $\sim 1/f$  spectra) produced good results.

## 2.6 Energy consumption and efficiency of photoreceptors

Reliability of photoreceptors responses has been traditionally measured with information rate estimates (Borst & Theunissen 1999). However, photoreceptors consume significant amount of energy in exchange of information transfer (Laughlin et al. 1998, Niven & Laughlin 2008). The energy consumption can be obtained from the ATP consumption of the Na<sup>+</sup>/K<sup>+</sup> -pump that keeps the ion concentrations in balance across the cell membrane, because most of the photoreceptor energy consumption is due to ion movements though the cell membrane either at rest or during signal generation. The Na<sup>+</sup>/K<sup>+</sup> pump has a stoichiometry of moving two K<sup>+</sup> ions (in) and three Na<sup>+</sup> ions (out) for one ATP molecule consumed. Normally the consumption is calculated from the potassium current by converting to an ion flux:

$$ATP\ consumption = \frac{I_K N_A}{2F} \quad (14)$$

where  $I_K$  is the total potassium current. The consumption is calculated from K<sup>+</sup> current instead of the Na<sup>+</sup> current because the reversal potential of K<sup>+</sup> is often known from measurements but the reversal potential of Na<sup>+</sup> often is not.

In case of photoreceptors the energy consumption can be divided to resting consumption in the dark, which is caused by the upkeep of the resting potential, and the consumption caused by signaling, which is the additional consumption during light compared to the resting consumption. The resting cost has been estimated to be circa 20% of the maximal consumption in the fly photoreceptors (Niven *et al.* 2007, Niven & Laughlin 2008). Photoreceptor consumption certainly depends on species, because the inherent leakiness (as whole cell resting resistance, for example) is very variable across different species. Those with fast vision tend to have higher energy consumption than slow vision species, for example the fruitfly (*Drosophila melanogaster*) compared to the urban bluebottle blowfly (*Calliphora vicina*).

The energy efficiency in photoreceptors is a measure that can be calculated from the relation of the information rate to the energy consumption, telling us how many ATP molecules are needed per 1 bit of information. Niven et al 2007 used four different fly species to compare their energy efficiency with white noise stimulation and a constant (average) contrast. They showed that the maximal information rate is related to maximal energy consumption.

### 3 Aims of research

The aim of this thesis is to study photoreceptors in a more natural state than has been done before by using novel algorithmical and computational developments. The thesis covers the following sub-projects, where I have aimed

1. to develop a method for estimating information rate in natural visual information flow, which generates nonlinearities and a response with a non-gaussian amplitude distribution in the photoreceptor signals. The method needs to include as little assumptions as possible.
2. to build and test a virtual environment for insects. The environment should cover a large part of the visual field of the animal with sufficient brightness and it needs to be fast enough for fast insect vision. It should also include a feedback control of the stimulus on the basis of the animal movement.
3. to investigate visual information transfer under dim conditions with a mathematical model. Model simulations are used to derive information rates that are then analysed to measure performance of photoreceptors under dim conditions.
4. to find out, what kind of voltage-gated  $K^+$  channels are most useful in photoreceptor membranes by simulating a database of different photoreceptor designs and their voltage responses for natural LIC. The channel composition is compared to information rate and energy consumption.



## 4 Summary of methods

### 4.1 Simulating photoreceptor excitable membranes

The excitable membranes of photoreceptors of two different insect species, the American cockroach (*Periplaneta americana*; Salmela 2012) in paper III and the fruitfly (*Drosophila melanogaster*; Vähäsöyrinki 2006) in paper IV, were simulated. The models were based on Hodgkin-Huxley type conductance parameters, which are determined in earlier publications (Hodgkin & Huxley 1952). The models simulate the photo-insensitive membrane from the LIC input to the voltage response of the photoreceptor, which can be described with a single differential equation (eq. 2). Other differential equations are then needed for voltage dependent  $K^+$  channels, whose activating and inactivating states are described as two-state models (eq. 4). Differential equations in the models are solved by using numerical integration of the fixed time step fourth-order Runge-Kutta method in Matlab environment (Mathworks), because of continuous input, current or LIC, prevents using any variable step solvers. Both stimulus types are expressed as vectors with time index, with step size the same as the numerical integration step.

### 4.2 Coarse simulation of the phototransduction cascade

Photoreceptor responses were simulated with a coarse model of transduction in paper III by using Poisson statistics (equation 1). Photon count during 1 ms bins was picked at random from a Poisson distribution. Parameter  $r$  (in eq.1) was varied as course of time by the stimulus, which was either white or pink noise with Gaussian amplitude distribution. Then each photon was replaced with a conductance bump template, which was either constant or varied within the experimentally found variation of the bump. The variation could be either by latency and/or by the amplitude of the bump. Finally the computed conductance was passed through the membrane model of the photoreceptor, with experimentally defined parameters.

### 4.3 Database of neurons

The properties of photoreceptors were analyzed by using a database of simulated responses in paper IV. A similar database method for studying effects of channels on cellular signalling has been used before in different models for neural system (Golowasch *et al.* 2002, Kispersky *et al.* 2012, Prinz *et al.* 2003), but in photoreceptors. The photoreceptor model of the fruitfly was stimulated with the LIC, which was estimated from voltage response for naturally stimulated photoreceptor. Maximal conductances of the model were varied, as based on a random distribution, which is logarithmically flat and which covers conductances from single channels to maximal experimentally determined conductance. The ion channels of the fruitfly included in the model were Shaker, Shab, novel, K<sup>+</sup> leak and the unspecific leak. The ratio between the two leaks was fixed, which prevented unnaturally high (or low) resting potentials. With every channel composition responses for the quiescent stimulus and the three different natural stimuli were computed. In the results, different conductance compositions gave different voltage responses for the same stimulus sequence. The responses were then analyzed by calculating information transfer and energy consumption for each simulated model and stimulus sequence. The results were finally presented in reference to the channel composition.

### 4.4 Shannon information capacity estimation

In papers I and III the naturalistic signals did not have Gaussian amplitude distributions, but, for comparison purposes the Shannon information capacity was also estimated, here from coherence function  $\gamma^2(f)$  (see e.g. Borst & Theunissen 1999):

$$\gamma^2(f) = \frac{|P_{xy}(f)|^2}{P_x(f)^2 P_y(f)^2} \quad (15)$$

where  $P_{xy}(f)$  is the cross spectrum between input and output, and  $P_x(f)^2$  and  $P_y(f)^2$  are power spectra of input and output time series, respectively. All spectra were estimated by using Welch method of dividing the time series in smaller, 50% overlapping sections and then the spectra of the corresponding sections were calculated individually and then averaged to obtain the final estimates.

Shannon information capacity is possible to derive from the coherence function by modifying equation 13 (Borst & Theunissen 1999):

$$C = - \int_0^{\infty} \log(1 - \gamma^2(f)) df \quad (16)$$

#### 4.5 Technical measurements of virtual environment

In paper II the construction, design and technical properties of the virtual environment were investigated. Irradiances and contrast of the virtual ball were measured with a spectrometer (USB4000 UV-VIS, Ocean optics Inc.) which was attached to a fiber optic cable (NA = 0.22). The irradiances were measured with the ball fully illuminated. The contrasts were determined using various sized black and white patches with opposite color background. The contrast was measured as the Michelson contrast between the patch and the background. Temporal properties of the virtual world stimulation were investigated with a photodiode. The data acquisition was done with 50 kHz sampling frequency.

## 5 Summary of the results

### 5.1 Estimating information rate of continuous signals

Paper I shows a method for estimating the information rate between two continuous signals. For this purpose we derive a relationship between mutual information and information rate. It was shown that between the input stochastic process  $\bar{X}_d = \{X_i | i = 1, 2, \dots, d\}$  with a length (d) and similarly for output  $\bar{Y}_d$ , the mutual information is linearly related to information rate:

$$I(\bar{X}_d; \bar{Y}_d) = Rd + c, \text{ when } d \geq L \quad (17)$$

where c is a constant depending on the initial conditions. This equation is constrained by the order of the Markov process L, which prevents using shorter series lengths than L. In practice mutual information  $I(\bar{X}_d; \bar{Y}_d)$  was estimated with varying lengths (d) and then a linear fit was done among the estimated mutual informations obtained.

The amount of data in the mutual information estimate is reduced by two methods for increased accuracy and reduced computation time. First cross mutual information function: CMIF(t) = I(X; Yt) (Schreiber 2000) was estimated, with t equal to the lag between input and output. Then the output is shifted back according to the lag of the maximum CMIF, so that there is no lag between the input and output that would reduce the needed L. Secondly, a PCA analysis was done for  $\bar{Y}_d$  and N highest PCA components were taken, which covers over 98% of the variance. N is kept constant among the varying d in mutual information estimates. Input  $\bar{X}_d$  is then multiplied by eigenvectors of the N highest PCA components of  $\bar{Y}_d$ . This typically reduced high dimensional mutual information estimate to 2-4 dimensional analysis per the input and output.

The method was validated with the known information rate estimate which is the Shannon information capacity estimate for a Gaussian input, which was convolved with a linear low pass filter and then a second varying Gaussian white

noise is added to the filtered signal. The information rate and the Shannon information capacity showed a one-to-one relationship up to circa 360 bits/s, after which the information rate degenerates to be smaller than the Shannon estimate. This can be corrected by increasing the length of the signals, but not needed in this work.

The novel method was used for two different kinds of datasets as examples: the visual processing of blowfly photoreceptors, and, for generality purposes, surface temperature data from US weather stations. For both datasets it was possible to estimate information rates, which in both cases were notably higher than the Shannon estimates, in agreement with the fact that the data was non-Gaussian and/or nonlinear.

## **5.2 Virtual reality environment**

Paper II presents the development of a virtual reality environment, which was based on image projection inside of a sphere in a computer controlled system. We found out that the biggest coverage of the sphere (65 %) can be achieved with a fish-eye lens attached to the DLP projector, whose colour wheel has been removed for fast black-and-white stimulation. A track ball was added to the system with a possibility of closed loop experiments between the track ball and the light stimulation. Computer control of the stimulation consisted of the actual 3D objects which can be moved, rotated etc. for making a stimulus sequence.

The virtual reality setup was validated rigorously with various tests which studied both spatial and temporal properties. The size of the pixels, which was between range of 0.75 - 2 deg for height and 0.75- 0.25 deg for width, is dependent on elevation of the projection (Paper II fig 2b). RMS pixel size which is calculated from simulations is near 0.5 deg, depending on the elevation, so the resolution in the sphere is restrained by pixel size in the lower part of the sphere. In the upper part the width of pixels is smaller than the RMS spot size. Pixel density changes also as a function of elevation, causing small variation of irradiance in the sphere. The maximum irradiance is near 4 W/m<sup>2</sup> in the central ring of the sphere, which corresponds to cloudy day in natural surroundings, and the maximum increases to be near 5 W/m<sup>2</sup> near the top of the sphere. The gamma of the projection is 2.56 and the contrast decreases as stimulus area increases, with a minimum of 0.2 Michelson contrast units.

Temporal properties of the sphere set-up was investigated with a light diode measurements. The projector works with 120 Hz refresh rate, which is divided in four sequences (circa 2 ms duration each): blue, green, red and white, which isn't computer controlled unlike the color ones. The DLP projector works with mirrors and so a varying irradiance is possible to achieve with varying a light pulse duration of each segment. Because the white is calculated from the color values, it gives a periodic deviation, which causes power spectrum of the light to have peaks at 120 Hz and its multiples. This problem can be easily solved by changing the projector in such a way that it only contains 3 colour channels. Similarly, power spectra of intracellular recordings of fast blowfly photoreceptors showed the 120 Hz peak and its harmonic components, which can be solved by changing to projector with only 3 colour channels.

To further test the usefulness of the virtual world set-up, cockroach behaviour was investigated with the track ball with closed-loop control. The environment was a 2 m times 2 m forest with thin trees. With slow photoreceptors (e.g. like those of the cockroach) the 120 Hz was not a problem even with the original design, because the high-frequency cut-off of photoreceptor signals takes place between 10-15 Hz. In the tests cockroaches avoided most of the trees and then began to follow the edge of the forest which has a sharp contrast between black outside and bright inside.

### **5.3 Photoreceptor performance at single photon level**

In paper III responses to discrete, single photon absorptions, so-called quantum bumps under very dim conditions were investigated by using cockroach photoreceptors and with measured and simulated data which was generated on the basis of photoreceptor properties, determined in electrophysiological experiments, and an electrical analogue model built on those principles. The simulations of single photon absorptions were done using a Poisson process, where the mean of the Poisson distribution is dependent on the stimulation.

We analysed visual information processing both using Shannon information capacity and the novel method developed in paper I. Variable light intensity was

used to change the properties of the input Poisson process. Analysis showed that in low intensity light ( $< 10$  ph/s) the quantum bumps of Poisson shot noise dominates information rate with both white noise and noise with  $1/f$  spectrum as the stimulus. At higher light levels a non-zero width of the latency distribution of the bump responses reduced the information rate significantly, but the width of the amplitude distribution did not have a notable effect. The Shannon information capacity fails to capture information correctly at low light levels and using it leads to overestimation of the effects of the latency distribution due the non-linear nature of the latter. There was no significant difference in information rates between the cell conductance and voltage signals. However the Shannon method gives lower information rates when light level is above  $10^5$  ph/s, because of the high nonlinearity in membrane voltage generation (self-shunting caused by the approaching reversal potential of the light-current, and the voltage-dependent  $K^+$  channels). Simulations overestimated information rates compared to recorded voltage responses, due to the lack of light adaptation when light intensity was below 1 ph/s, possibly because some information (in the computational sense) was coming from other sources than bumps or from bias in our estimates.

#### **5.4 Effect of $K^+$ channels to information transfer in photoreceptors**

In paper IV the effects of various types of voltage-dependent  $K^+$  channels for information rate were studied by creating a database of voltage responses, which originated from photoreceptor models with different channel composition but identical stimuli. The stimulus was a light-induced current (the LIC), which was estimated from intracellularly recorded voltage responses of fruitfly photoreceptors. The information rate had a maximum as a function of mean conductance, which is a sum of the mean voltage-active conductances and passive (leak) conductances. This maximum was dependent on the standard deviation of differentiated voltage and differentiated LIC; the differentiated voltage is almost linearly related to information rate in the database and higher standard deviation of the differentiated LIC stimulus gives higher maximal information rate in the database.

Although there is a maximum of information rate as a function of mean conductance the maximum was relatively wide. By taking 20 simulations with the highest information rates, the optimal composition of maximal ion channel

conductances is possible to examine. Figure 6 at paper IV shows steady-state conductances and channel compositions of the 20 cell models with highest information rates. The steady-state conductances are similar in shapes, because the so-called novel conductance dominates over the other conductances. The novel conductance has also a good scaling properties according to the stimulus, because it is the only voltage-dependent channel type here that does not inactivate. Other voltage activated  $K^+$  channels have smaller maximal conductances than the novel one, and the leak has a small range around 0.1 nS which results in a small resting consumption.



## 6 Discussion

### 6.1 Information rate estimation: methods and their relation to each other

The method that is developed in paper I gives the information rate between two continuous signals without assumptions which are typical for other methods. Traditionally, the method of choice for estimating the information rate is Shannon information capacity (Borst & Theunissen 1999, Van Hateren & Snippe 2001). However, it involves assumptions, which do not correspond to reality in studies of neural signals. The most erroneous problems in studies of neural coding are assumptions of Gaussian distribution of input and output signals and linear coding, which are not true in case of neural coding.

The assumptions of Shannon information rate have been averted by some investigators. Direct methods for estimating information rate were described in Strong et al 1998 for the action potential (pulse-) code, later expanded by Juusola and de Polavieja 2003 for continuously coded (analogue) signals. Both of those methods estimate the entropy rate of the average response and the entropy rate of noise and then calculate information rate by subtracting the noise entropy rate from the average entropy rate by assuming additive noise. These methods need necessarily a large amount of repetition of same short stimulus for averaging, which needs longer recording time compared to the presently developed method.

There is a certain degree of similarity between the novel method in paper I and the older method by Strong et al 1998 in estimating the information rate. They both estimate the rate from extrapolation fits in regards of inverse of time, marked as  $T$  in the Strong et al paper. By noting that their  $T$  corresponds to  $d$  in our method, we can see that dividing formula 17 by  $d$  and changing mutual information to entropy rate differentiation, we arrive to same formula, except that our formula is restricted to the  $L$ -order Markov processes. It can be argued that the restriction applies also the Strong et al method, because they use serial extension with only the linear term to derive the formula, without deeper studies. So taking the first term gives a correct estimate when  $T$  is longer than the order of the Markov process in both average and noise entropy rate estimates.

The major difficulty in obtaining the information rate is estimation of mutual information or entropy regardless of the method used. First, there is the problem of estimating mutual information without assuming any shape for the distribution

in one dimension (Khan et al. 2007, Kraskov et al. 2004, Pereda et al. 2005, Victor 2002). Generally all methods are prone to a systematic error of underestimating or overestimating mutual information, mainly because of the limited amount of data. Secondly, every dimension added to the estimation reduces accuracy of the estimate by increasing the overall volume of the estimation space.

In paper I two ways are presented in order to reduce computation cost. Firstly, signals are shifted in time so that they are as close as possible, which is a common procedure in non-linear analysis to reduce the data needed (Pereda *et al.* 2005). This is the same as introducing a predefined delay to analysis. Secondly, the PCA analysis is used to reduce dimensions in mutual information analysis. By keeping the number of PCA components constant the error in the multivariate mutual information estimate is also constant, which significantly improves this analysis. Further, it significantly reduces computing time of the mutual information estimate. PCA is widely used in the reduction of spatial dimensions in image analysis (Hyvärinen *et al.* 2009), but it has not been so common in reducing dimensionality of time-dependent analysis, such as frequency responses functions. Both these methods help with difficult multivariate mutual information estimation and they both reduce the computation time needed.

## **6.2 Information rates of insect photoreceptors**

Lack of good measures for information rate in the case of non-gaussian inputs and nonlinear systems has led to the development of more general ways to measure the information rate than Shannon information capacity (Juusola & de Polavieja 2003, Strong et al. 1998). In papers I and III I show that the difference between Shannon information capacity and the novel method as explained in paper I vary, depending on how well the Shannon information capacity fits the measurements. Generally, if the measured signal have a large variation, the error of Shannon capacity estimate is also large. High amplitudes of responses are quite probable, when using natural stimulation, like in the present works, and consequently the Shannon information capacity tends to underestimate the information rate. By

using natural stimulation it is possible to have significantly high information rates. This can be contrasted to experiments with white noise stimulation that gives almost non-existing responses in case of cockroach photoreceptors (Heimonen et al. 2012). In the other end, the information rates may also be limited with natural stimulation by the fact the natural stimulus has a near  $1/f$  power spectrum and so necessarily smaller power in high frequencies (Juusola & de Polavieja 2003).

In paper III various non-linear distortions were added to the light signal. These are latency noise of the quantum bumps, amplitude noise of the bump, and a nonlinear membrane filter. One main finding here was that the amplitude noise does not affect either the information rate or the Shannon information capacity at any light level. In bright light ( $> 1000$  photons/s) bump latency decreases both the information rate and the Shannon information capacity. However, the bump latency causes a significant underestimate in Shannon information capacity compared to the information rate, because the latency gives a non-linear component to the transfer function of the photoreceptor. Similarly, at the intensity of 105 photons/s Shannon information capacity underestimates the information rate when the voltage responses are studied.

### **6.3 Information transfer of photoreceptors in dim conditions**

Information transfer of photoreceptors at bump level has been studied before by looking at the occurrence of the bumps elicited by dim flashes (Dubs et al. 1981, Howard & Snyder 1983, Lillywhite & Laughlin 1979). Those results show that certain percentage of the flashes does not produce a bump according to the Poisson law. Previous studies also include examination of the properties of single bumps in terms of latency and amplitude. However, they have not dealt with bump properties during continuous stimulation, although that is a natural way in which vision operates. On the other hand, there are no studies of the information rate estimates in conditions, where the responses consist of single photon signals, due to lack of suitable methods. The smallest information rates calculated are in Heimonen et al 2012 with circa 2 photons/s. They show decreasing information rates as light gets dimmer, which has been reported earlier with higher intensities as a result of the Poisson process (Faivre & Juusola 2008, Niven et al. 2007).

Information rate of photoreceptors in dim light conditions is limited by the Poisson shot noise formed by light quanta. The limitation gives a constraint for following neural processes in dim conditions. So information rate of following neurons is limited by the information rates of the photoreceptors. This could be one way to measure how many photoreceptors are pooled to higher order neurons.

Although information rate is restricted in bump conditions by quantum nature of light, there is significant difference between information rates caused by bumps, depending on the properties of stimulation. Namely, pink noise produces a significantly higher information rate than the white noise. In real recordings it is visible that bump signals are masked by other sources of input, which cause voltage changes in voltage responses. But stimulus clearly correlates with the other sources, because information rate is higher than in the simulations. Whether the correlation is natural or artificial due to artefacts created in the recording process, remains to be determined.

#### **6.4 Effects of potassium channels to information rate of photoreceptors**

The method developed in paper I makes it possible to identify the optimum despite the non-linearities of the membrane, e.g. voltage-dependent conductances. Further on, it is possible to compare the effects of different kinds of non-linearities, which are generated by various  $K^+$  channel compositions.

The high conductance of the so-called novel gives in simulations the maximal information rate. This is simultaneously the same conductance that gives the maximal standard deviation of the differentiated voltage. Notably, next station for signal processing after photoreceptor signals is the synapse, which forms a kind of differentiation of the voltage signal in bright light conditions (Juusola et al. 1995). It can be postulated that by maximizing the information rate of the photoreceptor signal in this manner the synapse also gets maximal input from membrane.

Natural stimulation gives better functional range for the photoreceptor membrane than use of the traditional Gaussian white noise. Further, the

functionality of  $K^+$  channels is also closer to the natural stage, when they are dynamically being activated, inactivated and deactivated. Secondly, when natural stimulation is used instead of Gaussian noise modulated stimulation with fixed contrast, there is no connection between the mean light intensity, standard deviation of the voltage and the standard deviation of differentiated voltage. This helps to identify causes behind the maximal information rate.

## **6.5 Computer controlled stimulation accuracy**

Computer controlled virtual environments have become a feasible solution for studying vision, because of technological developments. In paper II a virtual reality setup was developed for insects with a large visual field and high temporal resolution. However, they suffer from various computational problems, because computer graphics are designed for human vision.

Measurements of the properties of the virtual environment with large visual angles give accurate indication of its performance regarding the potential for stimulation of the target neurons or animals. The setup performance is limited by the properties of computer control and the physical properties of light. The computer control is designed for human use, and so with fast eyes of the flies the control is limited for example by the refreshing rate of the projector serving as a light source. The basic clocks of normal computers have accuracies of 1 ms, which is a limiting factor. Limitations of physics are prominent in spherical virtual environments, because all light is being reflected from wall to wall, giving lower contrast of the stimulus than intended. This limit exists also in nature, however, as most natural contrasts are relatively small (Laughlin 1981, Ruderman 1994). Therefore, technical measurements of used stimulus are important to determine actual light levels which model sees.

The virtual environment offers a larger visual field than customarily used point or point-like stimulation methods. This is useful especially if higher order neurons of insects will be studied, because they are likely to pool signals from several ommatidia of the compound eye. The large field will also increase their performance and make them easier to identify functionally. This can be seen in e.g. Piironen et al 2012, where the virtual environment was utilized in conjunction with novel multi-electrodes to identify motion sensitive neurons. In that work the

combination of new type of stimulation, novel recording technique and suitable analysing methods provide fast, simultaneous detection and identification of the motion sensitive neurons in insect brain, which was not previously possible.

## 7 Conclusions

In this thesis more a natural state of insect vision during experiments is reached, giving better understanding of neural performance of the animals. This development allows further studies, which gives simultaneously a more complete picture of the function of neurons and the brain. Specific issues in this thesis were the following:

1. A relatively assumption-free method for estimating information rate is developed and tested. It shows a good agreement with the Shannon information capacity in case of Gaussian white noise input, linear filtering and a Gaussian white noise output. With the method it is possible to analyse many kinds of time-series, exemplified here with photoreceptor voltage responses and series of local surface temperature on different geographical locations. (paper I)
2. Spherical virtual environment was developed that offers a large visual field, necessary in studies of animals for covering their often large visual field. Using a computer controlled DLP projector gives fast and flexible possibilities to generate a varied light stimulus. Use of the trackball-system makes it possible to perform behavioural experiments with the virtual environment with tethered animals. (paper II)
3. A coarse computational model was created of photoreceptors for examining the light stimulus to membrane voltage transformation, ranging from dim to bright light. The model takes into account the quantum nature of light. Limit of the information transfer at the dim conditions ( $< 10$  ph/s) was confirmed to be photon shot noise. In addition, at higher intensities the latency distribution of bumps decreases information rate, but the amplitude noise does not. (paper III)
4. A database of voltage responses of fruitfly photoreceptors was developed with varying maximal conductances of several types of current, simulated using the same computationally estimated LIC stimulus. The database analysis shows a maximum of information rate as a function of mean conductance. The maximum is further identified with a definite composition of channels, namely a high novel conductance and small/average other conductances. This also co-occurs with relatively low energy consumption in the resting state. (paper IV)

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