Monte Carlo Simulation of Shot Noise Analysis for Reconstructing Elementary Events: Quantum Bumps in Photoreceptor Cells

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Shot noise analysis is a frequently applied method for reconstructing elementary events from experimental data of noisy signals. The subject of this paper is the reconstruction of single photon responses in photoreceptors, the so-called quantum bumps, from experimental data which have been obtained at high light intensities where the quantum bumps can no longer be observed as individual events. The application of this method requires some basic prerequisites which are very likely not satisfied in photoreceptor cells. This situation makes the results of the method questionable. In order to estimate the reliability of shot noise reconstruction in photoreceptors we directly compare the reconstruction results with well known input parameters by using a simulation model for the elementary mechanisms in the cell membrane which cause the response to light stimulation. The comparison shows that the reconstruction method starts to yield errors already at low intensities if only a few percent of the light controlled ionic channels in the cell membrane are open on average. The errors of the method become about 100 percent in the light intensity regime between 10 percent open channels and the onset of bump speck contact on the membrane. The anticipation that the reconstruction method yields single channel events at high light intensities is disproved at least for physiologically realistic intensities.

Introduction

An experimental method which is frequently used in order to investigate signal transduction in photoreceptor cells are *quantum bump measurements.* If stimulated either by steady state light at sufficiently low intensity or by light flashes at sufficiently low energy, many photoreceptor cells show electric responses, *e.g.* electric signal currents under voltage clamp, to the absorption of single photons. In this paper, we mainly refer to quantum bumps in the *ventral nerve photoreceptor of Limulus* (VNPL), but our conclusions will also be valid for bumps in other cells. Bump measurements at VNPL and the conclusions drawn from their results have been described in detail by Grzywacz and Hillman [1], Stieve [2], Keiper *et al.* [3], and Schnakenberg and Keiper [4].

If bumps are evoked by stationary light, their rate λ (mean number of bumps per time) depends linearly on the light intensity I [5]. This means that only at sufficiently low intensities I bumps can be observed as individual elementary events, *i.e.,* as transient deviations $b(t)$ of the signal current from its base line which are separated from each other as functions of time. We call this the bump regime of light intensity. In this regime, the bumps are evaluated in terms of typical parameters, *e.g.* rate λ, amplitude, duration, net charge transfer (time integral of the bump current), decay rates a.s.o. These parameters turn out to be fluctuating quantities which are described by histograms. From their mean values and their variances conclusions are drawn for the molecular mechanisms of transduction.

As the intensity I exceeds some value I_1 the bumps start to overlap and to melt together as functions of time such that their parameters can no longer be evaluated. Nevertheless, one would like to pursue the concept of bump parameters beyond I_1 since one is interested in conclusions for the transduction processes also at higher intensities, *e.g.* in order to locate adaptation mechanisms in the transduction process.

The first question which arises in this context is whether there still exists something like an elementary quantum bump beyond I_1. The generally accepted picture of a bump is that it is caused by transient openings of particular, so-called light sensitive ionic channels which lie within some

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bounded membrane region around the position of photon absorption. This membrane region is called *bump speck*. In VNPL bump specks are estimated to have a radius of a few μm (*cf.* [6]). Since in VNPL the total rhabdomere membrane region has a linear dimension of about 50 to 80 μm, it may well be that bumps exist as spatially separated events on the membrane although their signal responses as functions of time already melted together because the membrane is integrating the electrical events. This concept of an intermediate intensity regime where individual bumps exist, but are not observable as functions of time, is rather speculative although not unreasonable. It is this intermediate regime to which shot noise analysis is applied in order to reconstruct the bump parameters from the statistical properties of the fluctuating signal current which is assumed to be a superposition of elementary bumps (Wong [7], Wong and Knight [8], Wong *et al.* [9, 10]). This reconstruction method will be described in the following two sections.

The intermediate intensity regime, if it exists at all, will surely be bounded towards increasing intensities at some value I_2, at which the bump specks start to touch and to overlap. Beyond I_2, the bump picture breaks down conceptually. In order to make this point clear we introduce the concept of *signal transmitter molecules* which are assumed to be released after a photon has been absorbed, to diffuse along the membrane and to interact with the light-sensitive channels and thereby opening the channels. Such transmitter molecules have been identified in vertebrate photoreceptors and in other receptor cells, unfortunately not yet in VNPL. If the bump specks spatially overlap on the cell membrane, the light-sensitive channels receive opening signals from transmitter molecules which originate from different photon absorption events. This means that the concept of a bump as the response to a single photon becomes physically unrealistic.

The hazard of bump reconstruction by shot noise analysis is that the upper intensity limit I_2 of the intermediate regime is experimentally not accessible and that the reconstruction method nevertheless continues to deliver results, *i.e.* bump parameters, beyond I_2. We have pointed out this hazard already in earlier papers (Schnakenberg and Wong [11] and Schnakenberg [12]). In this paper, we present a Monte Carlo simulation of a shot noise experiment in order to elucidate the limitations of shot noise analysis quantitatively. In our simulations, the photoreceptor cell membrane is represented by a model system which includes transmitter release, diffusion, interaction with channels, opening and closing of the channels as described above. Different from the experimental situation, we now know both the input, *i.e.* rate and number of transmitter releases, parameters of free, *i.e.*, non-interacting bumps, and the output by shot noise analysis of the simulated noisy signal. Comparing input and output we can quantitatively assess the reliability of the method.

The results of our simulations indicate that the intensity regime for a meaningful application of shot noise analysis is very narrow. As mentioned above, however, shot noise analysis delivers results in terms of parameters of hypothetical elementary events at any intensity. It has been suspected that the elementary events resulting from shot noise analysis at high intensities are openings and closings of single channels [10, 12]. We shall show at the end of this paper that not even this expectation is realistic.

Campbell's Theorems and the Reconstruction Method

The statistical physical basis of the reconstruction of elementary events on the basis of shot noise analysis are *Campbell's theorems* (Campbell [13], de Felice [14], van Kampen [15]). Let $J(t)$ be a noisy signal which is generated by a superposition of *identical* and *statistically independent* elementary events $b(t)$, *i.e.*

$$J(t) = \sum_i b(t - t_i), \tag{1}$$

where the t_i are random times occurring at a mean rate λ. We assume that $b(t) = 0$ for $t < 0$, *i.e.*, $b(t - t_i)$ starts at t_i, and that $b(t)$ is a transient event, *i.e.*, $b(t)$ has a finite duration. Then Campbell's theorems say that

$$\langle J \rangle = \lambda \int_0^\infty dt\, b(t), \tag{2}$$

$$\langle (\delta J)^2 \rangle = \lambda \int_0^\infty dt\, b^2(t), \tag{3}$$

$$S(\omega) = 2\, \lambda\, |\tilde{b}(\omega)|^2, \tag{4}$$

where $\langle \ldots \rangle$ denotes the time average of the signal, $\delta J = J(t) - \langle J \rangle$, $\langle (\delta J)^2 \rangle$ is the variance of the signal $J(t)$, $S(\omega)$ it its spectral density and $\tilde{b}(\omega)$ is the Fourier transform of the elementary event $b(t)$:

$$\tilde{b}(\omega) = \int_0^\infty dt\, b(t)\, \mathrm{e}^{-\mathrm{i}\omega\mathrm{t}}. \tag{5}$$

The left hand sides of Campbell's theorems can be evaluated from the experimental data of the noise signal $J(t)$. Thus the right hand sides can be used to obtain information of the elementary events $b(t)$. One might even suspect that knowing the Fourier transform $\tilde{b}(\omega)$ of the elementary event $b(t)$ one might be able to reconstruct $b(t)$ completely, but this is not possible since the spectral density delivers only the absolute value $|\tilde{b}(\omega)|$ of the Fourier transform and the phase remains unknown. The usual way of evaluating the noise data is to assume a more or less plausible functional form for $b(t)$ and to determine its parameters. A very rough evaluation in this way is to assume that $b(t)$ has the form of rectangular pulses, *i.e.* $b(t) = A$ for $0 < t < d$ and $b(t) = 0$ otherwise. With this assumption for $b(t)$, A has the meaning of the amplitude and d that of the duration of the elementary events. We then have

$$\int_0^\infty dt\, b(t) = Ad, \qquad \int_0^\infty dt\, b^2(t) = A^2 d. \tag{6}$$

Note that the product $F = Ad$ delivers the correct value of the time integral of the signal $b(t)$. Evaluating $S(\omega)$ at $\omega = 0$ we also have from Campbell's theorems and the definition (5)

$$S(0) = 2\,\lambda\, F^2 = 2\,\lambda\,(Ad)^2. \tag{7}$$

Inserting the right hand sides of equations (6) and (7) into Campbell's theorems and solving for λ, A and d we obtain

$$\lambda_\mathrm{r} = \frac{2\langle J\rangle^2}{S(0)}, \quad A_\mathrm{r} = \frac{\langle(\delta J)^2\rangle}{\langle J\rangle}, \quad d_\mathrm{r} = \frac{S(0)}{2\,\langle(\delta J)^2\rangle}. \tag{8}$$

We have added a subscript r to the symbols λ, A, d in order to emphasize that the quantities on the left hand sides of (8) are reconstructed quantities which in general depend on the assumed functional form $b(t)$ for the elementary events. At present, λ_r and $F_\mathrm{r} = A_\mathrm{r} d_\mathrm{r}$ yield the correct values for the rate and the time integral of $b(t)$, respectively, *i.e.*, independent of the assumption for $b(t)$, but this will become different if the elementary events are considered to fluctuate, *cf.* next section.

Even if the true functional form of $b(t)$ is far from being a rectangular pulse, A_r and d_r as calculated from (8) will yield at least an estimate and the correct order of magnitude of the amplitudes and durations, respectively, of the true elementary events.

A somewhat more flexible and realistic evaluation is to assume that $b(t)$ has the form of a so-called Γ-*event*

$$b(t) = \frac{F}{n!\,\tau}\,(t/\tau)^n\, e^{-\mathrm{t}/\mathrm{r}} \qquad \text{for } t > 0 \tag{9}$$

and $b(t) = 0$ for $t < 0$. The parameter F is the time integral of $b(t)$ and the time parameter τ can be interpreted as the duration of $b(t)$. Inserting the Γ-event of (9) into Campbell's theorems one obtains the following reconstruction expressions for the rate λ_r and the parameters F_r and τ_r:

$$\lambda_\mathrm{r} = \frac{2\langle J\rangle^2}{S(0)}, \quad F_\mathrm{r} = \frac{S(0)}{2\langle J\rangle}, \quad \tau_\mathrm{r} = \frac{(2n)!}{2^{2n+1} n!}\,\frac{S(0)}{2\langle(\delta J)^2\rangle}. \tag{10}$$

The spectral density $S(\omega)$ of the Γ-event is obtained from the third of Campbell's theorems in the form

$$S(\omega) = \frac{S(0)}{(1 + (\omega t)^2)^{n+1}}\,. \tag{11}$$

At sufficiently high frequencies we obtain from (11) $S(\omega) \propto \omega^{-(2n+2)}$ such that n may be evaluated from plotting $\log S(\omega)$ as a function of $\log \omega$.

Shot Noise Analysis of Bumps in Photoreceptors

The shot noise reconstruction method described in section 2 is applied to photoreceptors by interpreting the noise signal $J(t)$ to be made up of the responses to the absorption of single photons, *i.e.* of quantum bumps, as the elementary events $b(t)$. If the measurements are performed under voltage clamp, the $b(t)$ are thus assumed to represent current bumps and their time integral F is the net charge transfer of the bump signal. The experimental information in terms of the mean current $\langle J\rangle$, its variance $\langle(\delta J)^2\rangle$ and its spectral density $S(\omega)$ is to be extracted from the fluctuating current $J(t)$ of the photoreceptor cell stimulated by steady state light.

The main problem when applying shot noise analysis in order to reconstruct quantum bumps in photoreceptors is that in general the bumps are neither identical nor statistical independent events as required by Campbell's theorems. Regarding the first requirement of identical events, we have mentioned already in section 1, that the bumps which are observed in VNPL experiments as individual events at low intensities (bump regime of intensity) fluctuate with respect to their amplitudes, durations, net charge transfers (time integrals), decay rates a.s.o. There is no reason why such fluctuations should be absent in the intermediate intensity regime where the bumps are imagined to be still individual events on the membrane but no longer separable as functions of time. We shall briefly point out possible implications of violating the conditions that the elementary are identical events. Let us denote the fluctuating elementary events by $b(t;\,\alpha)$ where α denotes the set of fluctuating parameters. Then Campbell's theorems can be generalized to yield

$$\langle J\rangle = \lambda\,\left\langle \int_0^\infty dt\, b(t;\,\alpha)\right\rangle_\alpha, \tag{12}$$

$$\langle(\delta J)^2\rangle = \lambda\,\left\langle \int_0^\infty dt\, b^2(t;\,\alpha)\right\rangle_\alpha, \tag{13}$$

$$S(\omega) = 2\,\lambda\,\langle|\tilde{b}(\omega;\,\alpha)|^2\rangle_\alpha, \tag{14}$$

where $\langle\ldots\rangle_\alpha$ denotes the average with respect to the set of fluctuating parameters α and $\tilde{b}(\omega;\,\alpha)$ is the Fourier transform of $b(t;\,\alpha)$, *cf.* (5). The reconstruction method is still based upon the reconstruction formulas (8) of section 2. Since the generalized Campbell's theorems contain the fluctuating event $b(t;\,\alpha)$ nonlinearly, the reconstruction method does in general not yield the averaged event $\langle b(t;\,\alpha)\rangle_\alpha$. In order to elucidate this point we insert the right hand sides of the generalized Campbell's theorems into the reconstruction formula for the rate λ_r in (8) and obtain

$$\frac{\lambda_r}{\lambda} = \frac{\langle F_\alpha\rangle^2}{\langle F_\alpha^2\rangle_\alpha}, \tag{15}$$

where λ_r is the reconstructed rate, λ is the true rate which enters into the generalized Campbell's theorems and F_α is the time integral of $b(t;\,\alpha)$. The value of the ratio λ_r/λ thus depends on the distribution of the time integrals F_α which in the case of bumps in VNPL is approximately exponential [1, 16]. For an exponential distribution, the right hand side of (15) is easily calculated to yield $\lambda_r/\lambda = 0.5$ which means that the reconstructed rate contains an error of about 50 percent.

The implications of violating the second requirement that the elementary events are statistically independent are much more serious and cannot be treated by some appropriate generalization of Campbell's theorems. The reason is that the errors of the reconstruction method applied to non-independent events depend on the kind of their interaction. We therefore present a model in the next section which gives a realistic picture of bumps interacting on the rhabdomere membrane of the photoreceptor cell. A computer simulation of shot noise analysis applied to this model will show us the limitations of reconstructing bumps from experimental noise data.

The Simulation Model

In our simulation model, the rhabdomere part of the photoreceptor cell is represented by a two dimensional quadratic array of a number of L^2 quadratic lattice compartments. The length h of the quadratic lattice compartments is chosen equal to the mean distance between two light-sensitive ionic channels on the real membrane, for VNPL approximately $h \approx 0.1$ µm, *cf.* [2, 3]. Neglecting the spatial fluctuations of the channel positions we assume that each of the lattice compartments contains exactly one light-sensitive ionic channel.

A single bump in the model is started by putting an initial number of T transmitter molecules into one of the lattice compartments which is chosen at random. We call the release of transmitter molecules in a lattice compartment a *transmitter source*. The transmitter molecules can diffuse on the membrane by jumping into neighbouring lattice compartments step by step. Each of the channels can reversibly bind a maximum number of K transmitter molecules as expressed by the scheme

$$C_k + T \rightarrow C_{k+1} \quad \text{transmitter binding} \tag{16}$$

$$C_k \rightarrow C_{k-1} + \mathrm{T} \quad \text{transmitter dissociation} \tag{17}$$

where C_k denotes the channel state with k transmitters bound, $k = 1, \ldots, K$, where K is called the channel cooperativity. The rate constants of the transmitter binding and dissociation in the above scheme are denoted by a_k and b_k, respectively. If a channel has bound the maximum possible number K of transmitters, it is counted as open.

The simulation is run by determining variable time steps Δt by making use of the so-called *minimal process method or Gillespie algorithm* which we have described in detail in an earlier paper (Fricke and Schnakenberg [17]). Then one of the transmitters is chosen at random which performs one of the following reaction steps:

1. If the transmitter chosen at random is free (not bound to a channel) it may diffuse to one of the

four neighbouring lattice compartments at a rate of $q = 4\ D/h^2$, where D is its diffusion, coefficient, or
2. bind to the channel in the lattice compartment where the transmitter stays at present at a rate of a_k if the channel is in a state C_k with $k < K$ or
3. is inactivated at a rate of μ.
4. If the transmitter chosen at random is bound to a channel in a state C_k with $k < 0$, it dissociates from the channel at a rate of b_k.

We have added step 3 to our model in order to account for the experimentally observed decay behaviour of the time course of bumps which can be expressed in good approximation by an exponential $\propto \exp(-\mu t)$ with a decay rate $\mu \approx 40\ 1/s$. A stimulation of the cell with steady state light will be simulated by starting transmitter sources repeatedly at times t_i which are chosen at random and independent of each other at a mean rate of λ which defines the input rate to be compared with the reconstructed rate λ_r from noise analysis.

Our simulation model is a minimal model for simulating bumps which interact on the finite area of the photoreceptor cell membrane and for studying the effect of this interaction on the results of noise analysis. It may be extended in many ways in order to include further experimentally established facts or meaningful suggestions in any specific photoreceptor cell. In particular, it seems very interesting to us to include into our model recent experimental results of Nagy and Stieve [18] and Nagy [19–22] in VNPL: three types of ionic channels are involved in the light response of the cell and very probably they are controlled by different types of transmitter molecules. We do not expect, however, that inclusion of different channel types into our simulation model and neither of other specific mechanisms will affect the essential conclusions from our simulations to be drawn in the next sections, namely a rather narrow limitation of the results of noise analysis. We even suspect that our conclusions will qualitatively also apply to vertebrate photoreceptors since interchanging open and closed states of channels will essential not influence the noise properties of the conductance of the cell membrane.

The values of the parameters which enter into our simulation are estimated from experimental data for VNPL. For the diffusion rate $q = 4\ D/h^2$ we choose $q = 1000\ 1/s$. With $h = 0.1\ \mu m$ (see above) this corresponds to a diffusion coefficient of $D \approx 0.25\ 10^{-7}\ cm^2/s$. This estimate takes into account that the diffusion constants of transmitter candidates in aqueous solution are expected to be of the order of $D \approx 10^{-7} \dots 10^{-6}\ cm^2/s$ and that for an effective two-dimensional diffusion along the membrane in a non-aqueous medium this value should be reduced by an order of magnitude, *cf.* [3]. As argued above, the transmitter inactivation rate is chosen $\mu = 40\ 1/s$. For the channel cooperativity K, we choose $K = 4$. This value is based upon the experimental finding that at high light intensities I the response in VNPL is $\propto I^n$ with an exponent $n \approx 3.3$ (Stieve and Schlösser [6]). In our earlier simulations with the same model as above (Fricke and Schnakenberg [17]) we have reproduced the experimental behaviour with a channel cooperative of $K = 4$. The rate constants for transmitter binding and dissociation are chosen as $a_k = 100\ 1/s$ and $b_k = 120\ 1/s$ independent of the channel states C_k. These values take into account the experimental results of Nagy [19] of a few *ms* up to about 10 ms for the open times of the three types of light sensitive ionic channels in VNPL. If one assumes that the transmitter molecules bind independently of each other to the channel, one would have $a_k = (K - k)a$ and $b_k = kb$ with some appropriate choise of the constants a and b. We have also tried this version in our test runs and did not find essentially different results compared to the version with equal a_k's and b_k's.

The linear dimension L of our lattice array is in principle irrelevant since the intensity is measured per area. There are physiological reasons, however, for a lower limit for L. Since we use helical boundary conditions in our simulations, L should be larger than a few bump speck radii. Assuming that the bump speck radius follows a diffusion law $r = \sqrt{2Dt}$ in usual units or $r = \sqrt{qt/2}$ in units of lattice compartments and inserting $t = d$ for the average life time of a bump, we obtain $r_{max} = \sqrt{qd/2}$ for the maximum bump speck radius. Inserting the above given value for q and $d \approx 76$ ms (see below) we have $r_{max} \approx 6$. Our standard choice for L is $L = 64$ such that our model membrane contains $L^2 = 4096$ ionic channels. Choosing larger values of L will not essentially change our results. Our input rate λ of transmitter sources should then be interpreted as a measure of the light intensity I nor-

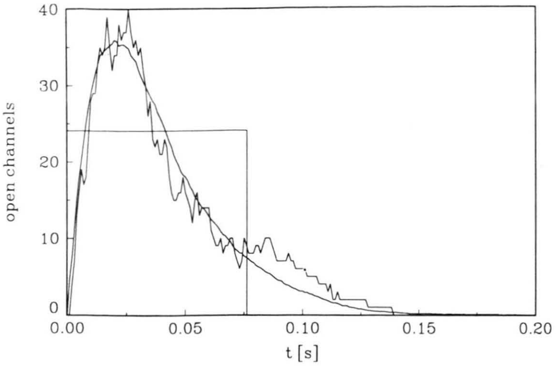

Fig. 1. Simulated bumps: single simulated bump (noisy curve), mean bump (smooth curve), equivalent rectangular pulse.

malized to this lattice size. At very high values of λ, we had to cut down our lattice dimensions to $L = 32$ since our computer capacity was limited. In this case, we have normalized the actual value of λ_{act} to the standard value by setting $\lambda_{act} = (64/32)^2\,\lambda$.

Finally, we have to determine the number T of transmitters which are released by a single transmitter source. With our above argued value for a maximum bump speck radius of $r_{max} \approx 6$ lattice compartments, we expect a maximum number of about 120 simultaneously opened channels. If each of the channels needs a number of $K = 4$ transmitters to be opened, we need at least about $T = 480$ transmitter molecules per source. Our choice for T in the following simulations is $T = 1000$. In view of the above estimates, this value seems to be reasonable, however, the above estimates are very rough and are nearer to a lower than to an upper limit for r_{max}. From the measured bump amplitudes of about a few nA and the conductances of the light-sensitive channels of about $20\,pS$ [18–20], one may equally well estimate $r_{max} \approx 1 \ldots 5$ µm which amounts to a number of 300 ... 8000 simultaneously opened channels for which 1200 ... 30,000 transmitter molecules would be needed. Such numbers would have gone beyond the capacity of our computer. We therefore keep to the value $T = 1000$ and interpret our simulation results in terms of relative quantities, *e.g.* as functions of the fraction of opened channels, instead of using absolute values.

We are now ready for starting simulations. Fig. 1 shows a simulated bump (noisy curve) obtained by activating one transmitter source. The smooth curve in Fig. 1 shows the mean bump for our above given set of parameters and the rectangular pulse is the equivalent rectangular event of the mean bump, *i.e.*, its amplitude A and its duration d are determined according to equations (6) by inserting the mean simulated bump for $b(t)$. The values of A and d are $A \approx 24$ channels and $d \approx 76$ ms. The latter value for d hits at least the order of magnitude of the experimentally observed bumps in VNPL. The shape of the mean bump in Fig. 1 qualitatively resembles that of experimentally observed bumps in VNPL, however, it lacks the initial convex phase since our model starts the transmitter sources instantaneously and not as a continuous function of time as should be assumed on biochemical reasons.

As pointed out above, the mean bump of Fig. 1 or its rectangular equivalent will serve now as the input and the reconstructed bumps will be compared to it in order to estimate the reliability of the reconstruction method.

Simulation of reconstruction

We start our simulations of the reconstruction method by stimulating our model membrane at an input rate λ of transmitter sources which each releases $T = 1000$ transmitters. Fig. 2 shows the mean number of open channels as a function of the input rate λ. This quantity should be considered the equivalent of the mean current $\langle J \rangle$ in the experiments. As to be expected, the mean number of open channels saturates at a value of $L^2 = 4096$ at high values of λ. The curve in Fig. 2 will serve us to translate the absolute λ-values into the equivalent

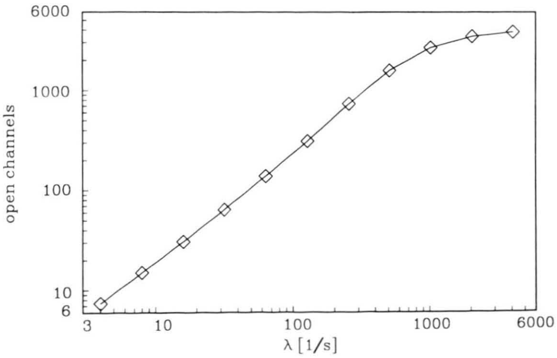

Fig. 2. Mean number of open channels as a function of the input rate λ.

fraction of open channels which is a relative quantity.

The first test of the reconstruction method is given in Fig. 3 which shows the reconstructed rate λ_r evaluated from the first of the Eqn. (8) as a function of the input rate λ in a double log plot. For $\lambda \leq 30$ 1/s we have $\lambda_r = \lambda$ within the statistical error due to the finite simulation time. In the regime between $\lambda = 30$ 1/s and $\lambda = 500$ 1/s the reconstructed rate λ_r is below the input rate λ by up to 30 percent, and for $\lambda \geq 500$ 1/s the reconstructed rate massively overshoots the input rate λ. It is interesting to translate these λ-values into fractions of open channels: according to Fig. 2 $\lambda = 30$ 1/s means a fraction of $\approx 60/4096$, *i.e.*, about only 1.5 percent open channels whereas $\lambda = 500$ 1/s means about 40 percent open channels. In other words, the reconstructed rate λ_r starts to deviate from the input rate λ if only a few percent of channels are open and it produces errors of orders of magnitude if half of the channels are open.

It is also interesting to compare the rate values at which λ_r starts to deviate from the input rate with that rate value at which the bump specks start to overlap on the membrane. If d is the mean bump duration we expect a mean number of $\lambda\ d$ simultaneous bump specks each of which has a radius of $r_{\max} = \sqrt{qd/2}$, *cf.* above. The bump specks will start to overlap as $\lambda d r_{\max} \approx L$ which yields a rate value of

$$\lambda \approx \frac{L}{d}\sqrt{\frac{2}{qd}}\,. \tag{18}$$

Inserting our above given parameters, we calculate ≈ 140 l/s from (18). This estimate tells us that the reconstruction methods produces unreliable values as soon as the bumps specks start to overlap. The result confirms our expectations in section 3.

Finally, Fig. 4 shows the amplitude A_r and duration d_r reconstructed from the second and third of the Eqn. (8) as a function of the fraction of open channels on a logarithmic scale. The solid horizontal lines are the values of the simulated mean bump as shown in Fig. 1. We again see that the reconstructed values start to deviate from the input values as soon as only a few percent of the channels are open. The deviations become massive for more than 10 percent open channels. In contrast to the behaviour of the reconstructed rate λ_r, the values of A_r and d_r at first overshoot the input values and then, for fractions of open channels beyond 50 percent, rapidly decrease.

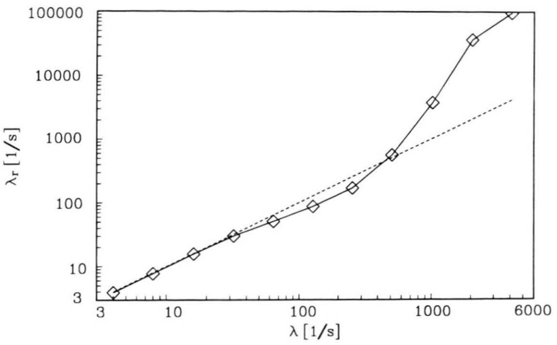

Fig. 3. Reconstructed rate λ_r as a function of the input rate λ in a double log plot.

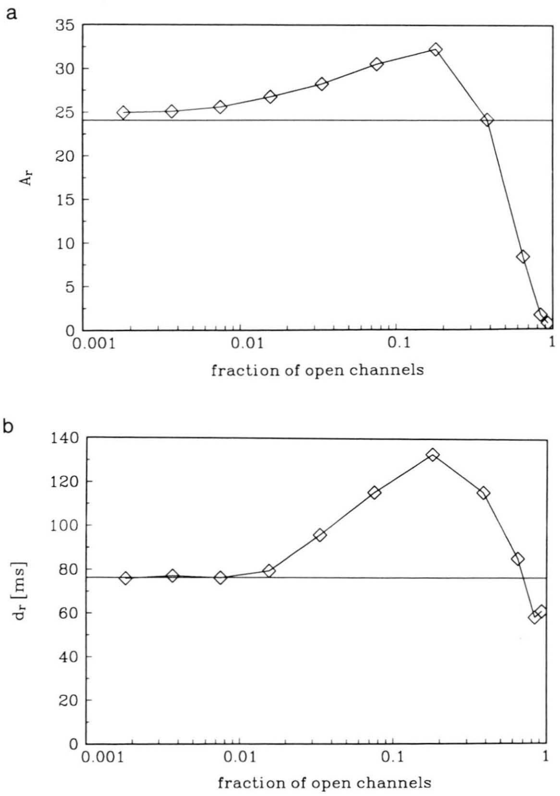

Fig. 4. Reconstructed amplitude A_r (a) and duration d_r (b) as a function of the fraction of open channels on a logarithmic scale.

A crucial shortcoming of our simulation model is the assumption that all transmitter sources release the same number of $T = 1000$ transmitter molecules. In contrast, the experimentally observed bumps in VNPL show a rather broad distribution of their amplitudes or their net charge transfers (time integrals) which suggests transmitter sources releasing variable numbers T of transmitters. The experimentally obtained histograms of bump amplitudes and net charge transfers can be approximated within the margin of the statistical errors by exponential distributions. This led us to repeat our above simulations with exponentially distributed numbers T of transmitters per source. If in a stationary light simulation of the cell a transmitter source is to be started at some time, the number of transmitters to be released was chosen according to a probability $p(T)$ given by

$$p(T) = \frac{1}{\langle T \rangle} e^{-T/\langle T \rangle} \tag{19}$$

where the mean number $\langle T \rangle$ of released transmitters was chosen $\langle T \rangle = 1000$ according to the discussion given above. The results of the simulations with variable T differed not very much from that with constant T. In particular, our estimates of the bounds for obtaining reliable results from the reconstruction method remain unchanged.

Single Channel Events

The reconstructed events which we obtained from our simulations in section (5) show a rapid decrease of their amplitude A_r and their duration d_r at high input rates λ, *i.e.* at high values of the mean fraction of open channels, *cf.* Fig. 4a and 4b. On the basis of this simulation result, one may suspect that the reconstruction method yields single channel opening and closing events at high intensities instead of one photon quantum bumps. We have tested this idea in two different ways and we received two negative answers.

Firstly, we have synchronously evaluated the spectral densities $S(\omega)$ of the total membrane area containing all L^2 channels and $S_1(\omega)$ of one particular single channel from our simulations. If the hypothesis that the reconstruction method yields single channel events were true we would expect

$$S(\omega) = L^2 S_1(\omega), \tag{20}$$

since the channels should contribute to the signal in an uncorrelated way. Fig. 5 shows a double logarithmic plot of $S(\omega)$ and $L^2 S_1(\omega)$ as functions of $f = \omega/2\pi$ at an input rate of $\lambda = 2048$ 1/s. According to Fig. 2, this λ-value corresponds to a fraction of about 80 percent open channels which in turn, according to Fig. 4a, corresponds to a reconstructed amplitude A_r of about 10 percent of the input amplitude. From Fig. 5 we see that $S(\omega)$ and $L^2 S_1(\omega)$ differ by at least an order of magnitude. Hence, the result of our first test of the single channel hypothesis is negative.

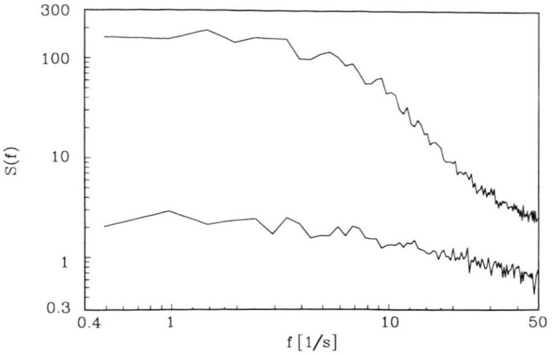

Fig. 5. Spectral densities $S(\omega)$ (upper curve) of the total membrane and $L^2 S_1(\omega)$ of one single channel as functions of $f = \omega/2\pi$ at an input rate of $\lambda = 2048$ 1/s.

In our second test of the single channel hypothesis, we evaluate the variance of the number of open channels from our simulation and compare it with a theoretical expression for the same quantity. This theoretical expression is derived on the assumption that the transmitter sources provide a spatially homogeneous but temporally fluctuating background of transmitters and that the channels open and close independently of each other by interaction with the transmitters. This is the situation that we expect if the reconstruction method yields single channel events. If the single channels hypothesis were true the two variances should coincide. Fig. 6 shows a double log plot of the ratio of the variances described above as a function of the input rate λ.

There is absolutely no λ-regime in which the ratio is at least approximately ≈ 1 and there is also no indication for an onset of an asymptotic behaviour of the ratio $\rightarrow 1$. This proves that the assumption of independent channel kinetics is not even satisfied for input rates λ at which the number of open channels begins to be saturated. Hence, the result of our second test of the single channel hypothesis is negative, too.

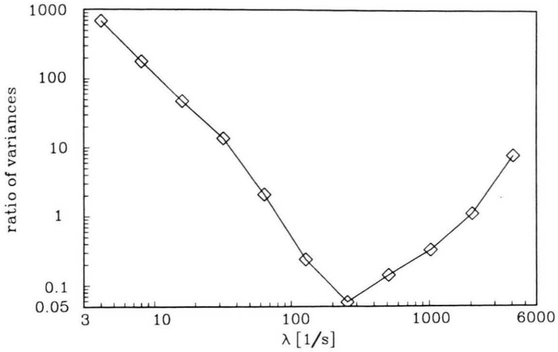

Fig. 6. Ratio of the evaluated and the calculated variance of the number of open channels as a function of the input rate λ for testing the single channel hypothesis.

It is clear that for sufficiently high values of the input rate λ the channels should become eventually independent. From Fig. 6 we estimate that this λ-regime is at least an order of magnitude beyond the onset of channel saturation. Note that the maximum value of λ in Fig. 6 is more than an order of magnitude above the λ-value for half saturation of the channels at $\lambda \approx 500$ 1/s. Translating this simulation result to VNPL, we expect independent channels at light intensities which are far beyond the physiological regime.

Conclusion

We have investigated the reliability of the shot noise reconstruction method for quantum bumps in photoreceptors by applying it to a computer simulation model. This model is a minimal description of what is expected to happen on the photoreceptor membrane at the end of the transduction chain. The details of the model and its parameter values have been chosen as to account for as many as possible of the experimental evidences for VNPL. The simulation model enables us to define all input values, in particular the mean quantum bump without interaction with other quantum bumps. This mean quantum bump serves as the standard for estimating the reliability of the reconstruction method.

Our simulations have shown that the reconstruction method starts to yield unreliable results for the reconstructed values of the rate λ_r, the amplitude A_r and the duration d_r, as soon as only a few percent of the channels are open on average. The errors of the reconstruction method arrive at 100 percent in the intensity regime between 10 percent of open channels and the onset of bump speck contact. On the other hand, the expectation that the reconstruction method yields single channel events at high intensities has been ruled out by our simulations at least for physiological intensities. The intensities at which the channels behave independently are estimated to be far beyond the physiological intensity regime.

The conclusion from our work is that quantum bumps in photoreceptors should be discussed only for light intensities at which they are observable as individual events as functions of time. All quantitative extrapolations to higher intensities seem to be uncertain and speculative. We doubt that the results of the reconstruction method have any physical or physiological meaning for light intensities at which the quantum bumps cease to be detectable as individual events.

It may well be that our simulation model is much too rough and too simple. It may even be unrealistic for a number of other photoreceptors than VNPL. Nevertheless, there is no general reason why a more complicated transduction mechanism including more than one transmitter type or more than only one channel type should behave simpler with respect to bump reconstruction by noise analysis. The essentials of our simulation model are the appearance of quantum bumps at sufficiently low intensities and channel saturation at high intensities. We think that these two essentials are realized in a very broad class of photoreceptors both for invertebrates and for vertebrates. From this point of view, bump reconstruction by noise analysis should be applied only if its most crucial prerequisite, namely the statistical independence of the elementary events, is guaranteed with absolute certainty.

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