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SHORT COMMUNICATION

A method for determining photoreceptor signal-to-noise ratio in the time and frequency domains with a pseudorandom stimulus

EERO KOUVALAINEN, MATTI WECKSTRÖM, AND MIKKO JUUSOLA
Department of Physiology, Biocenter and ORBIS Sensorius Oulu, University of Oulu,
Kajaanintie 52 A, 90220 Oulu, Finland

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Abstract

We have developed a method that utilizes repeated sequences of pseudorandomly modulated stimuli for calculation of the SNR either in the time or frequency domains. The method has the advantage that the distribution of SNR over relevant frequencies is readily observed. In addition, a SNR value, calculated as the ratio of the corresponding variances, is an estimate of the true SNR because it has been weighted by the cell's frequency response. The procedure offers significant advantages when studying signal transmission in nonspiking cells like photoreceptors.

Keywords: Nonspiking, Signal analysis, Vision, Contrast coding, Contrast sensitivity

Introduction

The signal-to-noise ratio (SNR) is needed to evaluate the performance of any signal transmission or processing unit, because it defines—with channel bandwidth—the information transmission capacity. Action potentials—once generated—are very efficient in information transmission in terms of SNR, because of their large amplitudes compared to any noise encountered in nerve axons. However, many cells do not generate action potentials, and the photoreceptors of various species offer good examples (see reviews, e.g. Järvilehto, 1979; Lamb, 1984). In nonspiking photoreceptor cells, noise is a serious limitation on information processing, because a major part of the noise is generated by the quantal nature of light and its absorption, which is also the basis of the voltage signals in photoreceptors (Fuortes & Yeandle 1964; Baylor et al., 1979; Lillywhite & Laughlin, 1979). The SNR has usually been estimated by applying flash or step-like stimuli with time averaging, while using various techniques to compare the averaged responses to the noise (e.g. Howard et al., 1987). However, this ignores one important feature of photoreceptors, namely the frequency dependence of their operation.

Insect photoreceptors can generally be treated as complex low-pass filters that acquire band-pass characteristics with increasing light adaptation (Järvilehto & Zettler, 1971; French, 1980*b*; Leutscher-Hazelhoff, 1975; Laughlin, 1981; Weckström et al., 1988; Juusola & Weckström, 1993). Therefore, a method that takes into account their frequency-dependent properties would give more accurate information about their performance. Here, we present a method based on stimulation with pseudorandom (white noise) modulated light. With this method it is possible to obtain a reliable measure of the SNR and, with time domain averaging, to estimate the SNR in the frequency domain.

Methods

Animals, light stimuli, and recording

In all experiments, we used wild-type adult blowflies (*Calliphora vicina*). Intracellular voltages were recorded from photoreceptor somata with glass capillary microelectrodes filled with 3 M KCl, and with resistances from 80 to 200 MΩ. The preparation and recording techniques are described in detail by Juusola (1993). The R1-6 photoreceptors were identified by criteria including an input resistance of about 30 MΩ and typical response properties—form, latency, and duration—(e.g. Järvilehto & Zettler, 1971; Hardie, 1979; Weckström et al., 1991).

Reprint requests to: Eero Kouvalainen, Department of Physiology, University of Oulu, Kajaanintie 52A, 90220 Oulu, Finland.

The light source was a green light-emitting diode (LED) (Stanley HBG5666X, with a peak emission at 555 nm), driven by a voltage/current converter. The light intensity level of the adapting background and pseudorandomly modulated contrast stimulus sequences were generated, controlled, and recorded by a computer (an IBM AT/486 compatible) using an ASYST (Keithley, Taunton, MA) based program and a 12-bit D/A converter (DT2821, Data Translation, Marlboro, MA). The noise stimulus was calculated as a series of random values using a Gaussian pseudorandom function of the ASYST, and stored for further use. The successive points of the stimulus sequences had negligible correlation. The low-passed, pseudorandomly modulated stimulus sequences had Gaussian amplitude distributions with constant power spectra up to 200 Hz (Fig. 1B), after low-pass filtering. The contrast of the light stimulus was defined as the ratio of the standard deviation (*SD*) and mean level (*M*) of the light stimulus sequence.

$$c = \frac{SD_{stim}}{M_{stim}} \tag{1}$$

Light contrasts from 0.03 to 0.5 were used in time-domain measurements. The light output of the LED was calibrated in terms of effective photons by counting the number of discrete responses evoked by single photons (Lillywhite, 1977) occurring during dim illumination after 60 min of dark adaptation. The resulting measure of intensity, the number of effective photon/s, was defined to be that which elicited, on average, the number of quantal events per second in the dark-adapted photoreceptor. The available intensity range was limited to the linear light output/current range of the LED and attenuated by neutral density filters (Kodak Wratten, Rochester, NY) to transiently cover more than 6 log intensity units. The lowest used adapting background was about 200 effective photons/s.

Recording procedures

After 90-s light adaptation to the selected background, the photoreceptor voltage response to the stimulus sequence was recorded with the microelectrode and a high-impedance preamplifier (SEC-1L, NPI Electronics, Germany). The recorded voltage and the monitored LED stimulus were led through a tunable filter (KEMO VBF/23 0.01 Hz–100 kHz low-pass dual channel elliptic filter, with 80-dB attenuation at 1.5 times the selected cutoff frequency) set at 500 Hz, and sampled at 2 kHz. Both voltage records were digitized with a 12-bit A/D converter (DT-2821, Data Translation, Marlboro, MA) and stored on the hard disk of the computer. The noise of the recording system was almost zero compared to the photoreceptor noise after filtering.

In the case of time-domain averaging and spectral analysis, the sampling process was initiated synchronously with the cycle of the pseudorandom noise signal (French, 1980*a*, Weckström et al., 1992) generated by the computer. Records of the stimulus (lasting 8 s) and the response were obtained during each cycle. A 6-s stimulus interval of mean steady background was allowed between every contrast sequence. After a preset number of stimulus sequences, the average response was calculated. The averaged data were then segmented for Fast Fourier Transform (FFT) analysis using a Blackman-Harris four-term weighting window with a 50% overlap of the segments (Harris, 1978). Spectrum estimates were calculated with standard FFT methods (Bendat & Piersol, 1971). To sustain a steady increase in light adaptation, the recordings were first executed at the lowest adapting background before proceeding to higher adapting backgrounds. After light adaptation, the cells were again dark adapted, and the recordings were rejected if the sensitivity and time courses of step responses did not return to their initial values.

Calculation of the SNR

Signal-to-noise analysis in time domain

It is first necessary to define some terms used in calculations: *Response* is the voltage *per se* recorded by the microelectrode as a function of time. *Signal* is that component of the response that is correlated to the modulatory stimulus and can, therefore, be separated from the response by time domain averaging. *Noise* comprises all of the components in the response other than the *signal*. In photoreceptor recordings, the noise contains at least the noise of the recording system (<50 μV), channel noise, and photon shot noise.

The photoreceptor SNR were calculated at different adapting backgrounds. Unlike step responses or sinusoidal modulation, the pseudorandom noise approach takes into account the signal and the noise at all frequencies simultaneously. The signal-to-noise analysis in the time domain was performed in the following way:

1. An adapting light background was turned on. Ten 2-s samples (with 2-kHz sampling frequency) of the photoreceptor voltage responses to that particular adapting background were recorded and used for calculating the variance of background-induced noise (σ_b^2).
2. A selected pseudorandomly modulated contrast signal was superimposed on the adapting background. The averaged variance of the contrast-induced voltage responses was calculated from ten 2-s samples (σ_c^2).
3. The variance of the photoreceptor signal (σ_s^2) was calculated by subtracting the variance of background-induced noise from the variance of the contrast-induced response that was recorded at the same adapting background.

$$\sigma_s^2 = \sigma_c^2 - \sigma_b^2 \tag{2}$$

4. The variance of photoreceptor signal was then divided by the corresponding variance of the background-induced noise to obtain a photoreceptor signal-to-noise ratio (SNR_{phr}).

$$SNR_{phr} = \frac{\sigma_c^2}{\sigma_b^2} \tag{3}$$

This represents the SNR because the variances of the photoreceptor signal and the background-induced noise represent the respective powers.

The adapting background was increased and the same procedure was repeated for each background used. The SNR was scaled to unit contrast by dividing the original SNR by the value of contrast used.

Signal-to-noise analysis in the frequency domain

In the case of a periodic input signal, time-domain averaging can be used to obtain a better estimate of the signal. As demonstrated by French (1980*a*), time-domain averaging can also be used when identical sequences of pseudorandom noise are applied as stimuli, as we have done in the present work. The averaged photoreceptor response, i.e. the photoreceptor signal, was used in two ways: for the calculation of the signal power spectrum, and for measuring the noise component (Fig. 1A). The latter was done by subtracting the averaged response (yielding the signal estimate) from the non-averaged responses (containing both signal and noise). This analysis, which could separate signal and noise power spectra, is illustrated in Fig. 2B. Signal-to-noise ratio (as a function of frequency) in the frequency domain was calculated by dividing the signal power spectrum by the power spectrum of the contrast-induced noise, and the result was also scaled to unit contrast (see above).

How reliable is the SNR estimate in the frequency domain?

The estimate of SNR we obtained with the method contains one source of error that could degrade the reliability of the experiments. Both the signal and noise estimates (σ_s^2 and σ_n^2) deviate from the true values; the estimates will include a residual noise, which is equal to the *n*th part of the initial noise power when *n* records are averaged. Thus, the SNR estimate has the form

$$SNR_{estimated} = \frac{\sigma_s^2 + \dfrac{\sigma_n^2}{n}}{\sigma_n^2 + \dfrac{\sigma_n^2}{n}} \tag{4}$$

where σ_s^2 represents the signal and σ_n^2 the noise variance, as explained above, and *n* is the number of the records averaged. This equation reduces to $1/(1+n)$, when the signal approaches zero. Thus with 30 averages, the limiting case of the SNR estimate has the value of 1/31 (= 0.0323). If the signal is considerably larger than the noise, we will underestimate the SNR by a factor of 0.969. If signal is equal to noise, the estimated SNR will be equal to the true value of unity.

The error in the SNR estimate can be reduced by increasing the number of averages. In addition, it is possible to remove this error by converting the estimated SNR values by

$$SNR_{true} = \frac{n+1}{n} SNR_{estimated} - \frac{1}{n} \tag{5}$$

which can be derived from eqn. (4) by setting σ_s^2/σ_n^2 to SNR_{true}, and rearranging terms. This correction does not change the values of the SNR appreciably when the signal is large (assuming *n* is large enough), but enables the investigator to obtain better estimates in case of a very low SNR, i.e. when the signal is small compared to the noise. How much better estimates are possi-

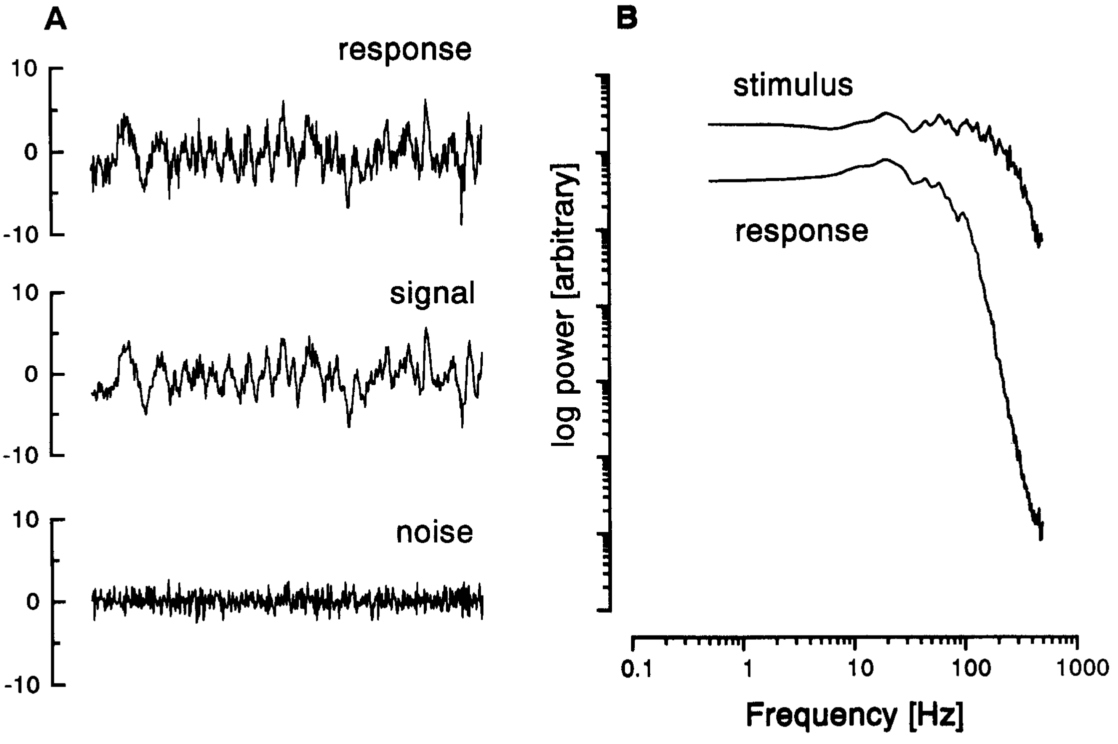

Fig. 1. Stimulating with a pseudorandomly modulated light contrast stimulus. A: Separation of noise and signal. The samples show a voltage response (top), the corresponding signal component (middle) obtained by synchronized averaging (30 times), and the noise component (bottom) as the difference between those two voltages. B: The power spectra of the pseudorandomly modulated light stimulus ("white noise") and a typical photoreceptor response to this stimulus. The adapting background was about 5.0×10^5 effective photons/s. Note that the input spectrum is approximately flat up to 200 Hz. Signals were filtered at 500 Hz (see Methods).

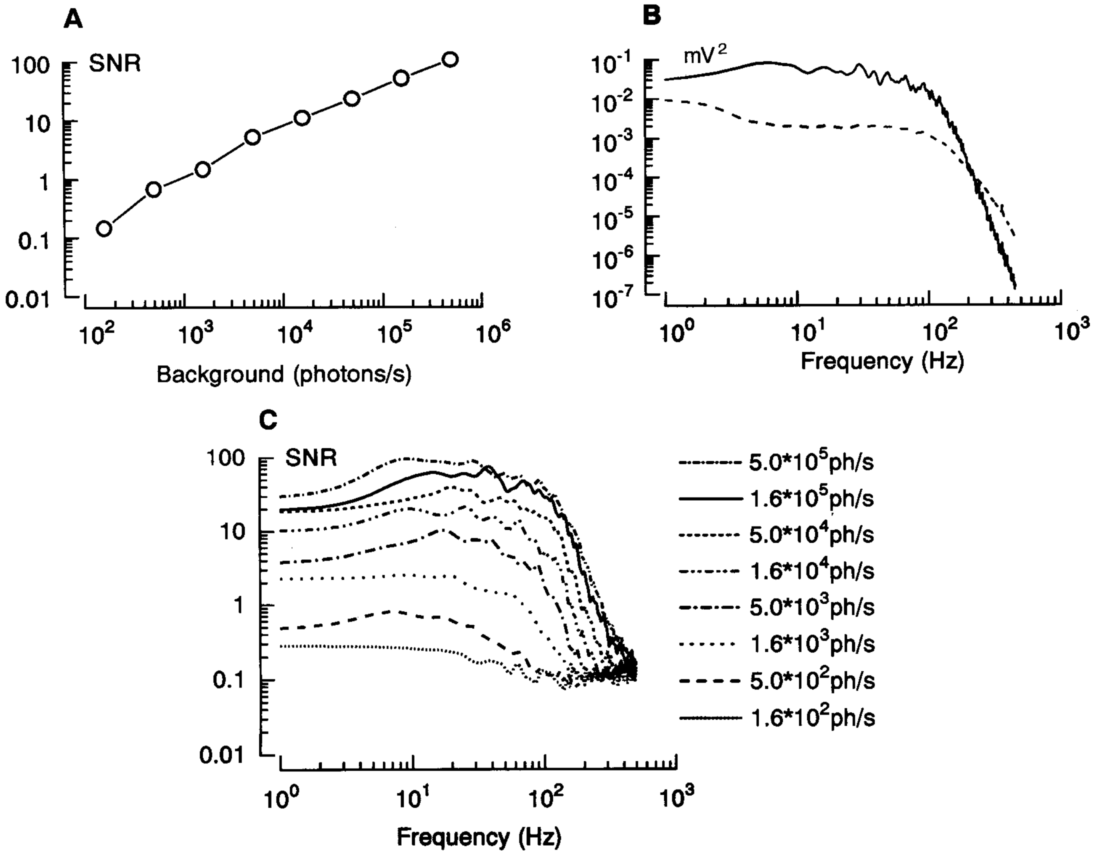

Fig. 2. Examples of results obtained with the described method. A: Signal-to-noise ratio of a fly photoreceptor determined with the pseudorandom stimulation method in the time domain. The ordinate is the SNR scaled to unit contrast as a function of the mean background intensity. B: Power spectra of the signal (continuous line) and noise (dashed line) components obtained with a background of 5×10^5 effective photons/s. The ratio of these is the SNR in the frequency domain (see Fig. 2C). C: Photoreceptor SNR in the frequency domain, obtained with different background intensities (indicated by the line key), with a contrast of 0.32, and scaled to unit contrast.

ble is limited by the reliability of the initial estimates of the power spectra (see e.g. Bendat & Piersol, 1971), whose evaluation is beyond the scope of this paper.

Results and discussion

Fig. 2 shows the SNR scaled to unit contrast (*a*, determined in the time domain, *c*, determined in the frequency domain) of the responses of a blowfly photoreceptor, determined at eight different background intensities ranging from 500 to 500,000 effective photons/s. The SNR was enhanced by increasing the light background (Fig. 2A). The SNR obtained with a background of 500 photons/s was ~0.7 but it increased to a value of 107 with a background of 500,000 effective photons/s. This agrees with investigations by Howard et al. (1987) who found a similar increase with *Lucilia* photoreceptors using step-like stimuli. Secondly, the SNR was frequency dependent, in the sense that it decreased sharply at high frequencies (Fig. 2C). This is probably a consequence of the fundamentally low-pass filter properties of photoreceptors, which reduces the responses to modulated intensity at high frequencies (see, e.g. Zettler, 1969; Järvilehto & Zettler, 1971; Leutscher-Hazelhoff, 1975; French, 1980*b*; Weckström et al., 1988). The SNR did not depend strongly on the stimulus frequency between 10–100 Hz. With the highest adapting backgrounds used, the SNR developed a shallow peak. This was conceivably a consequence of the resonance-like behavior of photoreceptor transduction, which is at least partially generated by the voltage-dependent membrane properties (Weckström et al., 1991; Juusola & Weckström, 1993).

The methods described here give important additional information about how cells process information. In the time domain, the method is simple because it does not require any spectral analysis, and gives a single value for the SNR in any experimental situation. The frequency-domain method requires the use of spectral analysis, but can be used to discover what kinds of signals and with what accuracy the cell responds? The SNR obtained with the frequency-domain method is an estimate that is biased but consistent, and the bias error decreases with the number of time-domain averages. The accuracy can therefore be increased at will within the limits of experimental accuracy.

The number of assumptions needed when applying this method is not large. We do not have to know any details of the processes under investigation. Particularly, no additional assumptions are needed in case of photoreceptors about the number of photons absorbed, or about the shape or nature of the single photon events. Even mutually dependent frequency components or nonlinearities do not render the method invalid, because the noise stimulus mimics the natural stimuli encountered by the animal. Thus, the voltage response to this noise stimulus contains all of the components that would be there also outside the laboratory. A number of investigators have analyzed the photoreceptor noise in order to characterize the "quantum bumps," or the voltage responses to absorption of a single photon, or the mechanisms of their summation (Dodge et al., 1968; Wong et al., 1982; Grzywacz et al., 1988, 1992; Juusola et al., in press). The method described in this paper, however, gives for the investigator the means to evaluate how much information is possible (for the photoreceptor, in this case) to be transferred to the synapse. No neural transformation except a process analogous to temporal or spatial averaging can recover the information lost in the noise. Many synapses have strong frequency selectivity (e.g. first synapse in the blowfly visual system: Laughlin et al., 1987), and thus the frequency-domain approach to the SNR is most appropriate.

Conclusions

It is clearly useful to determine the photoreceptor SNR with white-noise modulation as the stimulus. It enables the investigator to assess the performance of the cell in a way that takes into account the frequency response of the cell. The method yields a concise parameter, the SNR scaled to unit contrast. Analysis of the SNR in the frequency domain provides an additional technique that makes it possible to compare the SNR under similar conditions to those in which the noise and frequency response are usually determined. The generality of the method is in no way limited to a particular preparation or cell type, but may be applied to all problems where the efficiency of signal transmission is important.

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