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# On the statistical significance of electrophysiological steady-state responses

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**Abstract.** Steady-state stimulation is a useful paradigm in many physiologic and clinical situations, for ERG, Pattern-ERG and VEP. One of the advantages is the easy evaluation of the response via Fourier analysis. However, the question whether a given response is statistically significant or not has received little attention so far, although it is especially relevant in high noise, low amplitude recordings, as often occur in pathologic conditions. A given response is statistically significant if it is unlikely that its value is due to noise fluctuations. Thus appropriate estimates of noise and response are required. We have analytically derived formulas for the statistical significance of a given signal-to-noise-ratio $s$, based on two different estimates of noise: (1) Noise estimate by a 'no stimulus' recording, or by a '±average'. The former needs an additional recording, the latter can simultaneously be calculated as the standard average. (2) Noise is estimated as the average of the two neighboring spectral lines (one below, and one above the response frequency). Analytical solutions were obtained for both noise estimates that can easily be evaluated in all appropriate recordings. Noise estimate (1) performs much poorer than noise estimate (2), as can be seen from the following landmark values: Typical significance levels of 5%, 1%, and 0.1% require $s$ values of 4.36, 9.95, and 31.6 (1), and 2.82, 4.55, and 8.40 (2). The noise estimate based on the neighboring frequencies can be easily applied after recording, provided that the noise spectrum is reasonably smooth around the response and frequency-overspill was avoided. It allows a quantitative assessment of low responses in physiological threshold analyses and pathological conditions, e.g., 'submicrovolt flicker-ERG'.

**Key words:** ERG, signal-to-noise ratio, statistical significance, steady-state, VEP

## Introduction

The temporal presentation rate of visual flash or pattern stimuli is an important parameter in electrophysiological recordings. It evokes either transient potentials at slow presentation frequencies or steady-state potentials at high presentation frequencies. Transient and steady-state recordings differ in many respects:

- The temporal frequency may be used to isolate a response of a specific neuronal sub-population, e.g., the 30 Hz Ganzfeld-flash flicker stimulation exploits the physiological properties of the photoreceptors to isolate a pure cone-response [8].
- The rapid presentation rates lead to an increased neuronal activity per time interval compared to transient recordings, thus allowing a shortening of electrophysiological recording sessions.
- Different mathematical tools are used for data analysis. Slow presentation rates allow a detailed interpretation of positive and negative components and their temporal relationships, but noise intrusion is difficult to assess. When fast presentation rates are used (steady-state recordings), the corresponding responses of subsequent stimulations overlap considerably. For many experimental or clinical questions this is no serious disadvantage and is counterbalanced by the easy discrimination of signal and noise in the frequency domain, using Fourier analysis. As each stimulus presentation evokes a specific electrophysiological response, the recorded potential contains a spectral component at the stimulation frequency. This situation resembles classical resonance phenomena in mechanics like a pendulum or an oscillating string, where an oscillatory system is driven by an external force.

  Figure 1a shows an example of a 30 Hz flicker stimulus which evokes 16 responses in a time interval of 533 ms (left). An amplitude measure could be defined by averaging 16 peak-trough differences. However, it is much easier to take the Fourier component at the stimulus frequency of 30 Hz (Figure 1 right). Moreover, Fourier analysis allows an easy isolation of noise components. All components whose frequencies are no multiples of the stimulus frequency ('harmonics') can be regarded as 'pure noise'. However, the contrary is not true, as the Fourier component at the stimulation frequency is not a 'pure signal', but a superposition of signal and noise [11, 21]. This can be seen from Figure 1b, where the amplitude at 30 Hz is of similar amplitude as the neighboring noise components. Even if electrophysiological responses are recorded without visual stimulation we would expect a Fourier component at the stimulation frequency, which would represent a pure noise response in that case.

Steady-state recordings were first introduced by Regan [13, 14] who used analog amplifiers that were locked to the frequency of the visual stimulation. Since then the use of personal computers to record, average and store evoked potentials has enormously refined the numerical tools for data analysis. We

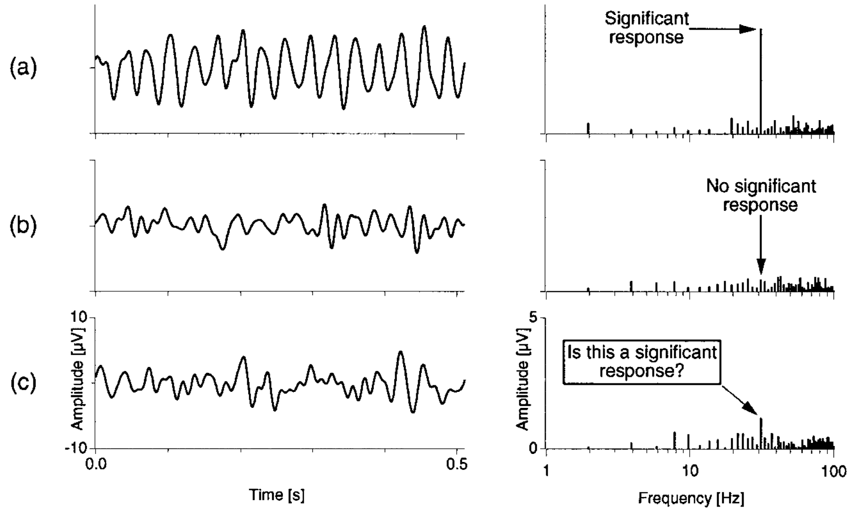


*Figure 1.* Significance related to the signal-to-noise ratio. Recording (a) shows a significant response of the visual system to a 30 Hz flicker stimulus. The 30 Hz Fourier component (right) clearly exceeds the noise level as defined by neighboring frequencies. Trace (b) is a pure noise response that was recorded under identical conditions as (a), but without visual stimulation. The Fourier spectrum indicates a non-significant response as the magnitude at 30 Hz is not larger than those of neighboring frequencies. Recording (c) shows a response under near-threshold conditions. In this paper we derived two mathematical formulas for the evaluation of statistical significance in such intermediate situations. In order to reach a 5% significance level, the 30 Hz component in (c) must exceed the average magnitude at the two neighboring frequencies in (c) by a factor of 2.82, or it must exceed the 30 Hz component of a control response like the one in (b) by a factor of 4.36.

no longer need to change the filter characteristics of amplifiers to perform a spectral analysis. Instead, software packages for Fourier analysis are widely available that allow an ‘offline’ isolation of the different spectral components in an averaged evoked potential. Unfortunately, these ‘FFT packages’ are often engineering solutions that do not take the special possibilities and problems of electrophysiological data into account. In the present paper we show that the statistical significance of steady-state potentials can be estimated easily in the Fourier domain. We will present simple formulas to estimate whether amplitude values in intermediate situations like the one in Figure 1c are significantly different from noise.

Statistical significance is usually evaluated by testing individual or group results against the data of a control group, e.g., the ISCEV standards recommend the recording of normal values for each recording condition [5, 7, 8]. Our approach differs in three respects. First, we did not collect separate electrophysiological data to derive these significance estimates. Instead, based on few assumptions concerning the distribution and spectral properties of the background noise, we derived these estimates through formal mathematical methods. Second, the resulting formulas are independent of the specific recording conditions and represent general statistical properties of steady-state recordings. They can be applied to any single steady-state response without requiring a pool of normal values. Third, we do not ask whether the magnitude of a response is significantly different from the mean value of a control group. Instead, we ask whether a response is significantly *different from noise*. Thus, our rules of thumb are best suited for measurements near threshold, e.g., for objective testing of visual acuity using the VEP [3, 22] or the 'submicrovolt flicker-ERG' [4].

The basic idea of our approach is to avoid an absolute criterion (e.g., $1\mu V$) for regarding a recorded potential as 'significant'. One problem in using such an absolute criterion is the very large interindividual variability of VEP and ERG amplitudes, even if the ISCEV guidelines and standards for clinical electrophysiology are followed. Likewise, the amount of residual noise which is present even after more than 100 trials may vary considerably between subjects. Thus we tried to relate statistical significance to the signal-to-noise ratio '$s$', which is a relative measure. As statistical significance corresponds to the probability to observe – simply by chance – a specific phenomena in the absence of any signal, we calculated the probability of a specific signal-to-noise ratio '$s_0$' in a pure noise response. While the magnitude at the stimulus frequency clearly defines the signal, there are different ways to estimate the noise level. We want to present mathematical formulas for two estimates where the noise is either derived from a control response or from the two neighbor frequencies of the stimulus frequency. The corresponding results differ considerably, not only in the numerical values and in the complexity of the formula, but also in their field of application. Fortunately, the scope of both solutions is largely complementary and covers most electrophysiological recording conditions.

One might wonder how we could define a 'signal-to-noise ratio' in a pure noise response, where no 'real' signal is expected. When analysing steady-state recordings the component at the stimulus frequency is usually refered to as 'signal', even if it should be denoted as 'signal & noise'. Consequently the correct name for a signal-to-noise ratio would be '(signal & noise)-to-noise ratio'. When deriving formula for evaluting the statistical significance we expect a pure noise response and the appropriate name would even be 'noise-to-noise ratio'. For simplicity, we will stick to the term 'signal-to-noise ratio'.

## Methods

*Fourier analysis of a pure-noise response*

In a ‘standard model’ of evoked potentials [14] the recorded VEP or ERG response $R(t_n)$ is a superposition of a signal waveform $S(t_n)$ and a noise waveform $N(t_n)$

$$R(t_n) = S(t_n) + N(t_n), n\epsilon[0, n_{total} - 1] \tag{1}$$

where $t_n$ denotes the time where the *n*th data point was taken and where $n_{total}$ is the number of datapoints in the recorded waveform. $R(t_n)$ may represent an average of several trials $(N_{trials})$. The term ‘trial’ does not imply that the time interval contains only one stimulus cycle. For steady-state recordings the responses evoked by repetitive stimulus presentations are usually recorded into a waveform of one ‘trial’. In Figure 1a the time interval of one trial (533ms) contains exactly 16 stimulus cycles. In the following we will show that for the application of our significance estimates a trial needs to be at least 10 times longer than the stimulus period. Furthermore, the analysis interval must contain an integer number of stimulus cycles to avoid ‘overspill’ of the response into neighboring frequencies.

In this model the signal $S(t_n)$ is assumed to be of constant waveform, while $N(t_n)$ varies over time as it reflects ‘internal’ noise sources like background EEG activity or eyemovement artifacts and the influence of ‘external’ noise sources like mains interference. As we are studying steady-state responses, we focus on the Fourier transform $\mathcal{F}_R(f_j)$ of the recorded signal $R(t_n)$ at those temporal frequency $f_j$ that was used for stimulus presentation (Figure 1)

$$\mathcal{F}_R(f_j) = \sum_{n=0}^{n_{total}-1} R(t_n) \cdot e^{2\pi i f_j n/n_{total}} \tag{2}$$

$\mathcal{F}_R(f_j)$ is a vector in a two-dimensional space with real part $x$ and imaginary part $y$

$$x = \sum_{n=0}^{n_{total}-1} R(t_n) \cdot \cos(2\pi f_j n/n_{total}) \tag{3a}$$

$$y = \sum_{n=0}^{n_{total}-1} R(t_n) \cdot \sin(2\pi f_j n/n_{total}) \tag{3b}$$

Fourier analysis of real data is, by necessity, always a discrete Fourier transformation (DFT). One special case of this with a fast algorithm is the

FFT, the Fast Fourier Transform. The discrete Fourier analysis does not yield a continuous spectrum, but rather discrete spectral 'lines'. Their position and spacing in the spectrum is directly related to the length of the time interval on which the DFT is based. The *j*th spectral line at the temporal frequency $f_j$ corresponds to *j* cycles in the time interval $T$, resulting in a frequency resolution of

$$\mathrm{d}f = \frac{j+1}{T} - \frac{j}{T} = \frac{1}{T} \tag{4}$$

Thus a time interval $T$ of 1 s will result in spectral lines at 1 Hz, 2 Hz, 3 Hz etc. A time interval of 0.5 s will result in spectral lines at 2 Hz, 4 Hz, 6 Hz etc. When the analysis interval $T$ contains an integer number $n_{cycles}$ of stimulus cycles with a length of $T_j = 1/f_j$, the frequency resolution can be expressed relative to the stimulus frequency $f_j$

$$\frac{\mathrm{d}f}{f_j} = \frac{1}{T} \cdot \frac{1}{f_j} = \frac{1}{n_{cycles} \cdot T_j \cdot f_j} = \frac{1}{n_{cycles}} \tag{5}$$

For $n_{cycles} = 10$ stimulus cycles per analysis interval the relative frequency resolution is 1/10 = 10%. When such a frequency resolution is used for the recording of 30 Hz flicker responses, the two neighboring frequencies of the stimulus frequency are located at 27 and 33 Hz.

Fourier-analysis packages often produce a 'power spectrum'. Unfortunately, this term is used in several meanings. It might simply be the square of the complex value at all frequencies, or it might be the convolution of this spectrum with the Fourier transform of an initially applied window function, resulting in a smooth spectrum. 'Windowing' the data may be appropriate in analysing ongoing EEG-data without external stimulation. However, it is *not* useful in the present context of evoked potential analysis, as we know the stimulus frequency and thus the response frequency. Furthermore, the analysis interval must contain an integer number of stimulus cycles to avoid 'overspill' of the response into neighboring frequencies. Thus a power spectrum does not appear useful to us: Windowing 'smears' the spectrum, and the squaring results in a measure that is not linearly related to peak-trough amplitude measures. The following analysis is based entirely on the magnitude spectrum and assumes that the analysis interval has been adequately chosen to avoid overspill. If the response is a pure sinusoid, the magnitude in the frequency domain is half the peak-trough amplitude in the time domain, as a sinus function of magnitude 1.0 covers the values from −1.0 to +1.0.

Due to the linear nature of the Equations (3a) and (3b), $\mathcal{F}_R(f_j)$ is a superposition of the Fourier spectra $\mathcal{F}_S(f_j)$ and $\mathcal{F}_N(f_j)$ of signal and noise.

$$\mathcal{F}_R(f_j) = \mathcal{F}_S(f_j) + \mathcal{F}_N(f_j) \tag{6}$$

This linearity holds for the vector representation of Fourier components. When nonlinear operations are performed to derive non-complex quantities like magnitude or power, signal and noise no longer superimpose linearly [11, 21]. Fortunately, the situation is simplified when the statistical significance is to be estimated, as we are calculating the probability for a specific response to occur without any signal $S(t_n) = 0$ in a pure-noise response

$$R(t_n) = N(t_n) \Rightarrow \mathcal{F}_R(f_j) = \mathcal{F}_N(f_j) \tag{7}$$

*Assumptions and rationale of the analysis*

We want to estimate the statistical significance of steady-state ERG or VEP responses in the pure noise response of Equation (7). Thus we have to make some assumptions concerning the spectral properties and the probability density of Fourier components at a specific stimulus frequency $f_j$.

First, it is essential to ensure that external noise sources like mains interference or monitor coupling do not interfere at the stimulus frequency $f_j$. Otherwise these contaminations would have a fixed temporal relation to stimulus onset and there would be no possibility to separate a signal from this kind of noise. Fortunately this problem can be solved by wisely choosing the stimulus frequency $f_j$ apart from known external noise frequencies. Second, we expect the remaining 'internal' noise of background EEG and eyemovement artifacts not to be time-locked to stimulus onset. When analysing the Fourier spectrum of a single trial, we might find typical frequency bands, e.g., the $\alpha$-activity in EEG recordings near 10 Hz. As the Fourier transform in Equation (6) is a linear operation, the Fourier transform of an averaged response of $N_{trials}$ trials is the vectorial average of $N_{trials}$ spectra. For a specific frequency $f_j$ both real part $x$ and imaginary part $y$ are the average of $N_{trials}$ individual real and imaginary parts. It follows from the 'central limit theorem' [12] that such an average of $N_{trials}$ random variables leads to a Gaussian probability distribution for $x$ and $y$ of identical width $\sigma$. This theoretical argument is validated by experimental findings [9], showing that the individual real and imaginary parts of a complex Fourier component are uncorrelated and of equal variance. Thus we assume the following distribution

$$f_N(x, y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}} \tag{8}$$

to give the probability to find a complex Fourier component of real part $x$ and imaginary part $y$. The width $\sigma$ the Gaussian distribution depends both on the amount of EEG background noise $\sigma_0$ in a single trial and on the number of trials $N_{trials}$ that were averaged to yield a specific ERG or VEP response.

As the noise is not time-locked to stimulus-onset, the resulting EEG-noise of subsequent trials is uncorrelated and $\sigma$ follows a '$1/\sqrt{N}$'-law

$$\sigma = \frac{\sigma_0}{\sqrt{N_{trials}}} \tag{9}$$

The rationale of the further analysis is based on a probabilistic approach. Equation (8) is the main assumption concerning the statistical properties of a noise component. The two-dimensional probability distribution of Equation (8) for the Fourier component is determined by a single parameter $\sigma$ which gives the amount of noise at a specific frequency $f_j$. Thus the problem consists in inversely estimating $\sigma$ from single Fourier components that share the same magnitude distribution. This can be done in different ways and the resulting formulas strongly depend on the chosen noise estimate. We will present two estimates of the background noise which lead to two signal-to-noise ratios $s'$ and $s''$.

- $s' = \frac{m_j}{n'_j}$ is the ratio of the magnitude of interest $m_j$ ('signal') to the magnitude of a control condition $n'_j$ ('noise') at the same spectral line $j$ (Figure 2a). This requires either a separate recording under conditions where no electrophysiological response is evoked (e.g., without visual stimulation) or the calculation of a '±average' [15, 18], where the responses to subsequent stimulations are averaged with alternating sign, which cancels the signal and leaves a pure noise response.
- $s'' = \frac{m_j}{n''_j}$ is the ratio of the magnitude of interest $m_j$ ('signal') to the average magnitude $n''_j = \frac{m_{j-1}+m_{j+1}}{2}$ ('noise') at the two neighboring spectral lines $j - 1$ and $j + 1$ (Figure 2b). Compared to $s'$ the following additional constraints must be fullfilled to apply $s''$
  - As the noise is taken from the same recording as the signal, we have to ensure that the two neighboring frequencies do not contain any residual signal. Steady-state components are narrow-band signals with a width of less than 0.01 Hz [6, 20]. As the resolution of a Fourier spectrum $df$ is related to the temporal duration of the recording $T$ via $df = \frac{1}{T}$ (Equation (4)), the length of the averaged recording should be significantly shorter than 100 s. When an analysis interval of more than 100 s is used, the two noise frequencies should not be the direct neighbors. As the frequency resolution of the Fourier analysis is very fine in that condition, it will be easy to find Fourier components outside the 0.01 Hz range (e.g., 2 or 3 spectral lines apart from the stimulus frequency) that may serve as noise estimates. If this constraint is fullfilled and if overspill is avoided, two neighboring frequencies can be regarded as pure noise components.

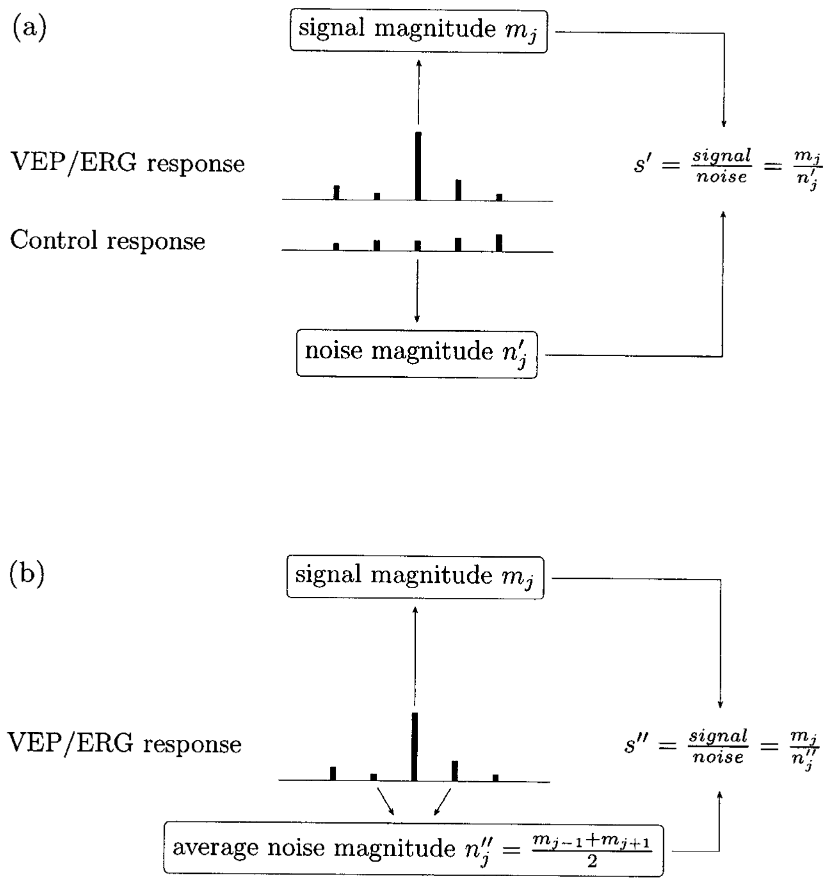


*Figure 2.* Two definitions of the signal-to-noise ratio $s$. The statistical significance of a steady-state VEP or ERG component can be derived from the signal-to-noise ratio $s$. While the magnitude at the stimulus frequency clearly defines the signal, there are different ways to estimate the noise level. The noise magnitude is defined either (a) as the magnitude $n'_j$ of a control response at the same temporal frequency, or (b) as the average noise magnitude $n''_j$ at the two neighboring frequencies.

- As the noise is estimated from slightly different frequencies, the noise spectrum must be coherent enough so that $n''_j$ is an unbiased noise estimate for the temporal frequency $f_j$. This requires a fine spectral resolution d$f \leq 1$ Hz resulting in a recording length of $T \geq 1$ s. However, this rule of thumb depends on the temporal frequency of the Fourier component of interest. As the time and frequency domain are usually described in logarithmic units, a resolution of 1 Hz may be appropriate for analysing a 30 Hz component, while it may be too coarse in the neighborhood of a 8 Hz component. Thus we would suggest a spectral resolution of 10% or less of the response frequency (i.e., for a 16 Hz response the spectral resolution should be 1.6 Hz or better, see Equation (5)). When evaluating the 'true' Fourier magnitude from a superposition of signal and noise as in Equation (6), Norcia et al. used one spectral line 2 Hz apart from the stimulus frequency to derive the noise level [11]. They additionally proposed to use two frequencies, one above and one below the stimulus frequency, to get a more accurate estimate of the noise at the stimulus frequency, whenever the EEG spectrum has a significant slope. We used this noise definition as the starting point to evaluate the statistical significance of $s''$.

In typical steady-state VEP and ERG recordings where the recording length $T$ is larger than 1s and where $T$ contains an integer number of stimulus cycles, all constraints can be fullfilled. Thus $s''$ can be applied more widely compared to $s'$ as no additional recording of a separate control response is required. This allows even a retrospective analysis of steady-state recordings where such an analysis was not intended at the time of the recording.

The analytical derivation of significance estimates from steady-state responses consists in calculating different probability distribution functions $f_X(x)$. $X$ denotes a specific quantity (like magnitude $M$), $x$ is a specific value of that quantity (like a specific magnitude value $m$) and $f_X(x)$ is the probability to find $x$ in the ensemble of $X$-values in a pure noise response. The distributions $f_X(x)$ are derived by calculating joined probabilities of two other quantities, e.g., when we are asking for the probability distribution $f_S(s)$ of the signal-to-noise ratio $s$, we first have to calculate the joined probability of a signal with magnitude $s \cdot n_j$ and a noise magnitude $n_j$, followed by an integration of this joined probability across all possible values of $n_j$. While the corresponding mathematical solution is rather simple for $s'$, it is much more complicated for $s''$. Thus only the resulting formula will be given in the results section and the detailed mathematical steps can be found in the section 'Appendix I'. Two figures illustrate the rationale and the resulting probability distributions for $s'$ (Figure 3) and for $s''$ (Figure 4).

## Results

### *Magnitude distribution of a pure noise response*

As a first step we have to find the probability distribution $f_M(m_j)$ for the magnitude $m_j = \sqrt{x^2 + y^2}$ of a complex Fourier component with real part $x$ and imaginary part $y$. To simplify the following calculations we introduce

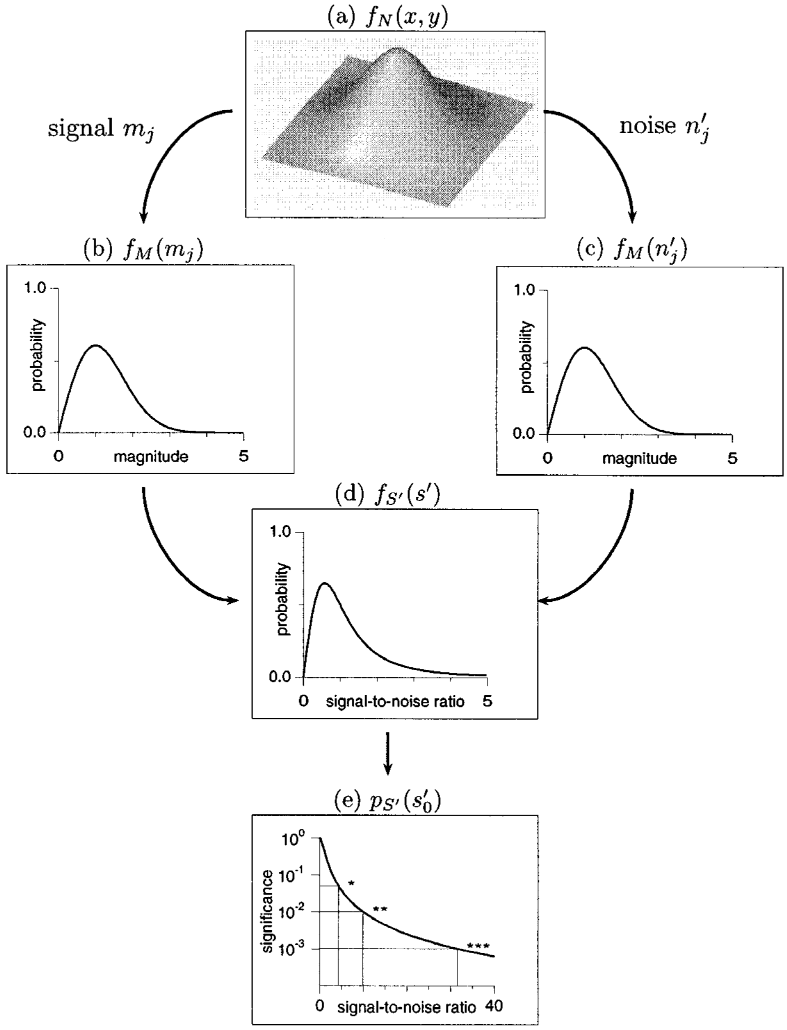


*Figure 3.* Significance $p_{S'}(s'_0)$ derived from $s'_0$. The signal-to-noise ratio $s'$ is here defined as the magnitude ratio of the VEP/ERG response at spectral line $j$ to the magnitude $n'_j$ of a control response at the same temporal frequency (Figure 2a). The signal (b) and noise (c) magnitude are taken from the same 'Rayleigh'-magnitude distribution $f_M(m_j)$ of a pure noise response (a). The distribution $f_{S'}(s')$ for the signal-to-noise ratio (d) allows the calculation of significance values $p_{S'}(s'_0)$ (e) as a function of the signal-to-noise ratio $s'_0$.

polar coordinates of magnitude $m_j$ and phase $\varphi_j$. As the entire mathematical problem may be scaled by the width of the Gaussian distribution $\sigma$, we expressed all real and imaginary parts (and thus all magnitude values) in multiples of $\sigma$. The resulting distribution $f_M(m_j)$ for magnitude values in

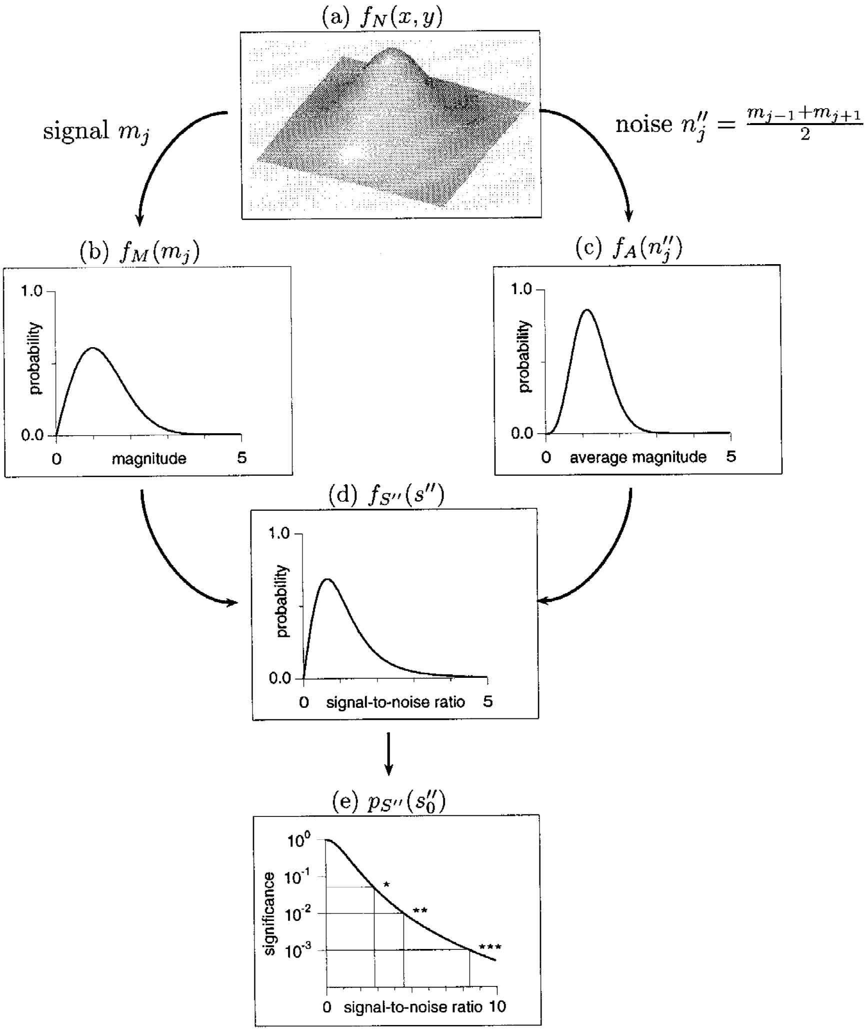


*Figure 4.* Significance $p_{S''}(s''_0)$ derived from $s''_0$. The signal-to-noise ratio $s''$ is here defined as the magnitude ratio of the VEP/ERG response at spectral line $j$ to the average noise magnitude $n''_j$ at the two neighboring frequencies (Figure 2b). The signal (b) and noise (c) magnitudes are thus taken from different distributions $f_M$ and $f_A$. The distribution $f_{S''}(s'')$ for the signal-to-noise ratio (d) allows the calculation of significance values $p_{S''}(s''_0)$ (e) as a function of $s''_0$.

a pure noise response (Figure 3b, c and Figure 4b)

$$f_M(m_j) = m_j \, e^{-\frac{m_j{}^2}{2}} \tag{10}$$

is a *Rayleigh* distribution and does not depend on $\sigma$ (Appendix I, Equations (22) to (27)). In this first step the phase information is lost as we have integrated the two-dimensional probability distribution of Equation (8) for components that have the same magnitude but arbitrary phase values.

*Noise estimate by a control response*

In evaluating the statistical significance we expect both the signal magnitude $m_j$ and the noise magnitude $n'_j$ of an appropriate control response to share the same magnitude distribution $f_M(m_j)$ (Figure 3b, c). The probability distribution $f_{S'}(s')$ for the signal-to-noise ratio $s'$(Figure 3d) is thus determined by the joined probability of $f_M(m_j)$ and $f_M(s' \cdot m_j)$ integrated over the entire magnitude range for $m_j$ (Appendix I, Equations (28) to (30)).

$$f_{S'}(s') = \frac{2s'}{(1 + s'^2)^2} \tag{11}$$

The statistical significance $p_{S'}(s'_0)$ (Figure 3e) is the probability to find a signal-to-noise ratio $s'$ beyond a specific value $s'_0$. It can thus be derived by integrating Equation (11) for all values beyond $s'_0$ (Appendix I, Equations (31) to (32)).

$$s'_0 = \frac{m_j}{n'_j} (\text{noise estimate } n'_j \text{ from control response}) \tag{12a}$$

$$p_{S'}(s'_0) = \frac{1}{1 + {s'_0}^2} \tag{12b}$$

Fortunately, this formula is very simple. Thus we can easily illustrate that it has sensible asymptotical properties. For very small signal-to-noise ratios $s'_0 \to 0$ the significance $p_{S'}(s'_0)$ approximates 1:

$$\lim_{s'_0 \to 0} p_{S'}(s'_0) = 1 \tag{13}$$

As the probability to exceed very small $s'_0$-values in a pure noise response is nearly 1, they correspond to statistically non-significant responses. For very large signal-to-noise ratios $s'_0 \to \infty$ the significance $p_{S'}(s'_0)$ approximates 0:

$$\lim_{s'_0 \to \infty} p_{S'}(s'_0) = 0 \tag{14}$$

Thus large $s'_0$-values correspond to small $p_{S'}(s'_0)$-values and indicate statistically significant responses. To illustrate the application of Equation (12b) for intermediate $s'_0$-values we have compiled some 'landmark' values for the

*Table 1.* Noise estimated by a control response, examples. When the noise estimate $n'_j$ is taken from a control response at the same temporal frequency as the signal magnitude $m_j$ (Figure 2a), then the statistical significance $p_{S'}(s'_0)$ can be easily related to a specific signal-to-noise ratio $s'_0 = \frac{m_j}{n'_j}$ by Equation (12b). This table shows some values that are typically used for significance estimation

| Signal-to-noise ratio $s'_0$ | Significance $p_{S'}(s'_0)$ |
|---|---|
| 4.36 | 0.05 |
| 9.95 | 0.01 |
| 31.6 | 0.001 |

signal-to-noise ratio $s'_0$ in Table 1 that correspond to commonly used significance values. Additionally we present the formula of Equation (12b) in Excel notation in the section 'Appendix II'.

Keeping in mind that no further assumptions about the spectral resolution or about the spectral width of an electrophysiological response are required in deriving Equation (12b), the simplicity of the resulting formula might lead to the impression that we have solved the problem. However, it requires a control response, which may often not be evaluable. Furthermore, while a $\pm$-response can be averaged simultaneously, the recording of a control response requires additional time and identical recording conditions. Thus we searched for a more general solution which could be applied even without recording a control response. In this case the noise magnitude has to be estimated from the same spectrum as the signal magnitude. As described in the Methods section we chose the average magnitude of the two neighboring frequencies as noise estimate.

*Noise estimate by averaging two neighboring magnitude values*

The mathematical steps to relate the statistical significance $p_{S''}(s''_0)$ to a specific signal-to-noise ratio $s''$ are very similar to the derivation of Equation (12b). The joined probability of the noise magnitude $n''_j$ and of the signal magnitude $m_j = s'' \cdot n''_j$ are integrated across the entire magnitude range for $n''_j$. This allows the calculation of significance values $p_{S''}(s''_0)$ by integration. However, there is an important difference between these two derivations. While the noise magnitude $n'_j$ was taken from the same 'Rayleigh' distribution for magnitude values $f_M$ as the signal magnitude, we have to consider the probability distribution $f_A(a_j)$ for the average $a_j$ of two magnitude values in a pure-noise response. This distribution can be derived from $f_M$ in Equation (10) by calculating the joined probability to find a magnitude value of $m_{j-1}$ for the left frequency and a magnitude value of $m_{j+1} = 2a_j - m_j$ for the right frequency (Appendix I, Equations (33) to (35))

$$f_A(a_j) = e^{-2a_j^2}\left(2a_j + e^{a_j^2}(2a_j^2 - 1)\sqrt{\pi}G(a_j)\right) \tag{15}$$

where $G$ denotes the Gaussian error function

$$G(z) = \frac{2}{\sqrt{\pi}}\int_0^z e^{-t^2}\mathrm{d}t \tag{16}$$

To evaluate the probability distribution $f_{S''}(s'')$ (Figure 4d) for the signal-to-noise ratio $s'' = \frac{m_j}{n''_j}$, the joined probability of $f_A(n''_j)$ and $f_M(s'' \cdot n''_j)$ is integrated across the entire magnitude range for $n''_j$ (Appendix I, Equations (37) to (39))

$$\begin{aligned} f_{S''}(s'') = &-\frac{2s''\left(s''^4\sqrt{\frac{1}{2+s''^2}} - 2s''^2\left(2\sqrt{\frac{1}{2+s''^2}} + \sqrt{2+s''^2}\right)\right)}{(2+s''^2)^{\frac{3}{2}}(4+s''^2)^2} \\ &+ \frac{8s''\left(6\sqrt{\frac{1}{2+s''^2}} + \sqrt{2+s''^2}\right)}{(2+s''^2)^{\frac{3}{2}}(4+s''^2)^2} \\ &- \frac{s''\sqrt{2}\left(-4+s''^2\right)\arctan(\sqrt{\frac{2}{2+s''^2}})}{(2+s''^2)^{\frac{5}{2}}} \end{aligned} \tag{17}$$

Surprisingly, this rather confusing Equation (17) becomes somewhat easier after the final step which relates the statistical significance to a specific signal-to-noise ratio. We finally find the following formula for the statistical significance $p_{S''}(s''_0)$ (Figure 4e) when integrating Equation (17) for all signal-to-noise ratios beyond $s''_0$ (Appendix I, Equations (40) to (41)):

$$s''_0 = \frac{m_j}{n''_j}(\text{noise estimate } n''_j = \frac{m_{j-1} + m_{j+1}}{2}) \tag{18a}$$

$$p_{S''}(s''_0) = \frac{2 + 4\frac{1}{2+s_0''^2}}{4 + s_0''^2} - \frac{\sqrt{2}s_0''^2\arctan(\sqrt{\frac{2}{2+s_0''^2}})}{\left(2+s_0''^2\right)^{\frac{3}{2}}} \tag{18b}$$

*Table 2.* Noise estimated by averaging two spectral neighbors, examples. When the noise estimate $n''_j$ is a result of averaging the magnitude values at the two neighboring frequencies (Figure 2b), then the statistical significance $p_{S''}(s''_0)$ is related to a specific signal-to-noise ratio $s''_0$ by Equation (18b). This table shows some values that are typically used for significance estimation

| Signal-to-noise ratio $s''_0$ | Significance $p_{S''}(s''_0)$ |
|---|---|
| 2.82 | 0.05 |
| 4.55 | 0.01 |
| 8.40 | 0.001 |

This final formula still looks complicated, but similar to Equation (12b) for $p_{S'}(s'_0)$ it has sensible asymptotic properties. For small $s''_0$-values, $p_{S''}(s''_0)$ approximates 1:

$$\lim_{s''_0 \to 0} p_{S''}(s''_0) = \frac{2 + 4 \cdot \frac{1}{2}}{4} = 1 \tag{19}$$

As the probability to exceed very small $s''_0$-values in a pure noise response is nearly 1, they correspond to statistically non-significant responses. For very large signal-to-noise ratios $s''_0 \to \infty$ the significance $p_{S''}(s''_0)$ approximates 0:

$$\lim_{s''_0 \to \infty} p_{S''}(s''_0) = 0 \tag{20}$$

Thus large $s''_0$-values correspond to small $p_{S''}(s''_0)$-values and indicate statistically significant responses. To illustrate the application of Equation (18b) we have again compiled some 'landmark' values for the signal-to-noise ratio $s''_0$ in Table 2 that correspond to commonly used significance values. Additionally we present the formula of Equation (18b) in Excel notation in the section 'Appendix II'.

## Discussion

Steady-state VEP and ERG recordings allow an easy discrimation of the electrophysiological signal from noise as all non-harmonic frequency components can be regarded as noise. The Fourier component at the stimulus frequency is thus a handy and non-ambiguous measure of neuronal activity which does not require any decision about where to identify the positive and negative peaks. This advantage is most obvious when analysing small responses near threshold (e.g., at low contrast) or in pathological conditions. While it may appear difficult to detect a small signal in the time domain (Figure 1c, left), the corresponding Fourier component may indicate a supra-threshold response (Figure 1c, right). However, the near-threshold responses in Figure 1b, c illustrate that the Fourier component at the stimulus frequency contains both signal *and* noise. Thus not the absolute magnitude value of a Fourier component determines its reliability, but the degree by which it exceeds the noise level. In this paper we derived two formulas to estimate the statistical significance of a steady-state response from the signal-to-noise ratio. The noise magnitude can be defined either by the Fourier component of a control response (Figure 2a) or by the average magnitude of the two neighboring frequencies (Figure 2b).

The two solutions differ not only in their resulting formula but also in their field of application. While averaging the components at the two neighboring frequencies for $f_A(a_j)$ can be done with any Fourier spectrum of a steady-state response, the magnitude of a control response for $f_M(m_j)$ requires either an additional recording under conditions where no VEP or ERG is evoked [5], or the averaging of a '$\pm$-response' where the trials are averaged with alternating sign. The additional recording time and the $\pm$-averaging feature of the recording software may not always be available. Thus we clearly favour to average two neighboring frequencies, which can even be applied to a retrospective analysis of steady-state recordings where a statistical test was not intended at the time of the recording. There are some minor restrictions concerning the temporal duration $T$ of a single trial which is related to the frequency resolution of the Fourier spectrum via $1/T$. To ensure that the spectrum around the signal component is coherent enough, $T$ should be larger than 1 s. In typical steady-state recordings this can easily be fulfilled by averaging trials over this sweep length.

What about a combined testing under conditions where both formula can be applied? There may be situations where one test gives a non-significant result (e.g., $p>0.05$) while the other test indicates a significant response (e.g., $p<0.05$). Does this indicate an inconsistency in our formula? We are dealing with processes that are random by nature and that can only be described by probability distributions. The two statistical tests use different noise estimates and are thus independent. Consequently, such 'inconsistent' results are expected to occur occasionally and do not indicate any formal inconsistency. However, when both tests are performed, a frequent mistake in multiple statistical testing should be kept in mind, which consists in choosing the 'best' significance value of a series of tests. This leads to an over-estimation of the statistical significance and can be avoided, for example, by Bonferroni correction.

However, there might be recording situations in which a different combination of the two tests may be the best choice. Suppose that we recorded a steady-state response with a high frequency resolution near $\mathrm{d}f = 0.01$ *Hz* where the neighboring frequencies might possibly contain some residual signal and cannot serve as noise estimates. Besides deriving a noise estimate from the stimulus frequency $f_j$ of a control response using Equation (12b), we could also calculate the average magnitude of the two neighboring frequencies of a control response using Equation (18b). This would combine the advantage of a better noise estimate when averaging two noise magnitude values with the advantage of using a control response where no residual signal component contaminates neighboring frequencies.

It might be confusing to find two different solutions for a single problem. But although we have only one signal component at the stimulus frequency we can find numerous noise components in the neighborhood of the signal or in a control response. We could have defined the noise level either as the average of 4 neighboring spectral components instead of 2 or as the average magnitude at the stimulus frequency and its two neighbors in the control response. Each of these definitions would lead to a different probability distribution for the signal-to-noise ratio and to a different formula that relates the signal-to-noise level to statistical significance. Here we described only two solutions, namely those that are most easily derived analytically and that can be applied most widely due to a minimum number of constraints. Adding more than the two neighboring frequencies into the calculation to get a more accurate estimate of the noise level may be advantageous. However, the assumption of a coherent noise spectrum may be violated when the spectral range of the neighboring frequencies is too large. Furthermore, pilot numerical calculations showed strongly diminishing returns on an increased number of neighbors.

The problem to detect a sinusoidal signal in a background of Gaussian noise applies not only to electrophysiology, but also to different contexts, e.g., to the field of electrical engineering. The statistical properties of the superposition of a sine wave and random noise were studied by Rice [16] which helped to estimate the ‘true’ magnitude of superposition of signal and noise in electrophysiological recordings [11]. An analytical approach that is closely related to our significance estimates can be found by Robertson [17]. He calculated the performance of an envelope detector that operated on a sine wave embedded in narrow-band Gaussian noise. When varying the signal-to-noise ratio and the number of averages he derived ROC-curves that related the detection rate for a present signal to the false alarm rate of an absent signal by varying the threshold value. Alberhseim [1] found a mathematical expression which condensed the 14 multicurve graphs of Robertson [17] into a single function and which had an accuracy of less than 0.2 dB over a wide range of parameters. We found similar results when comparing our formulas with those of Alberhseim [1]. The aim of our approach was to derive some simple rules that can be easily applied to steady-state responses. Thus we tried to bridge the gap between the more theoretical field of signal analysis and the practical field of evoked potential recording in vision science and clinical application.

Formulas (12b) and (18b) are designed to test whether a specific response is different from noise. However, they can also be applied to test whether two recordings are significantly different. When two responses $R_1(t_n)$ and $R_2(t_n)$ were recorded under identical stimulus conditions, then Formulas (12b) and (18b) can be applied to the Fourier spectrum of the difference response $R(t_n)$

$$R(t_n) = R_1(t_n) - R_2(t_n) \tag{21a}$$

$$\mathcal{F}_R(f_j) = \mathcal{F}_{R_1}(f_j) - \mathcal{F}_{R_2}(f_j) \tag{21b}$$

Equation (21b) contains a vectorial difference of the complex Fourier components for every frequency $f_j$. The magnitude spectrum of $R(t_n)$ can thus be treated using Equations (12b) and (18b) to test whether the two recordings $R_1(t_n)$ and $R_2(t_n)$ are significantly different.

The formulas (12b) and (18b) do not take into account the phase information but simply use the magnitude spectrum of a response. Phase information is related to the latency of a response and there might be experimental situations in which some phase values of a signal are more likely than others, e.g., when responses to checkerboard patterns are studied starting from contrast values well above threshold and going to contrast values near and below threshold the resulting responses will change monotonically. A response with phase values similar to those obtained for supra-threshold conditions will be regarded as more ‘significant’. Thus when additional information about the expected phase of a signal is available, it might be possible to refine the statistical analysis. Victor and Mast developed a statistical test (‘$T^2_{circ}$ statistic’) where the two-dimensional scatter of the Fourier component of repeated measurements is used to calculate confidence limits of steady-state responses [23]. This approach exploits the relationship between the real and imaginary parts of a complex Fourier component and consequently has a higher statistical power than the present analysis.

We did not take into account the phase information for two reasons. First, there is considerable interindividual phase variability in steady-state data. For some subjects the response to a specific stimulus shows more than one positive and negative peak, leading to higher harmonic components in the Fourier analysis. While the latency of the first peak in the time domain may be similar to those of another subject, the phase of the corresponding component at the stimulus frequency will be different. Thus the phase of a specific response near threshold can only be predicted when additional supra-threshold measurements were recorded for the same subject. Second, there are situations in which phase values change dramatically when reaching threshold stimulus conditions, e.g., when flicker VEPs or ERGs are recorded for red/green stimuli near equiluminance, a small shift in the red/green luminance ratio may inverse the stimulus conditions [2, 10], leading to dramatic phase shifts. For an arbitrary steady-state recording there might not be enough information to benefit from phase information. Thus our approach provides a general statistical test which can be further refined, e.g., by the $T^2_{circ}$ statistic in situations where information of the expected phase of a signal is available.

The approach of Victor and Mast [23] differs from our analysis in an additional aspect. The '$T^2_{circ}$ statistic' requires an analysis of the raw electrophysiological data, while our formula may be applied to the traces of averaged trials. Of course, non-averaged data preserve more information on the characteristics of the underlying noise than averaged data. Thus the '$T^2_{circ}$ statistic' offers an elaborate tool to test for statistical significance. A similar tool to study the cycle-by-cycle variance of steady-state responses was recently presented [19]. This method also requires an analysis of the raw electrophysiological data. Any laboratory that has the facilities to compute results based on a complete raw data set should use it. We here offer a solution to the real-world problem of averaged traces. Such kind of data is the basis of analysis in many laboratories, and to assess significance with a less-than-optimal method is always better than not to do it at all.

## Appendix I

Our goal is to assess the statistical significance of an electrophysiological steady-state response having a Fourier magnitude $m_j$ at the spectral line $j$. The rationale of our analysis and some notations are given in the Methods section. We here present the detailed steps to relate statistical significance to the signal-to-noise ratio. The derivations were based on the following steps

1. We consider the distribution $f_M(m_j)$ of one spectral magnitude $m_j$.
2. We estimate the distribution $f_{S'}(s')$ for the signal-to-noise ratio $s' = \frac{m_j}{n'_j}$
3. The resulting distribution $f_{S'}(s')$ is integrated to estimate the probability for a specific signal-to-noise ratio $s'_0$ to be reached by chance. According to the mathematical definition of statistical significance, this yields the significance $p_{S'}(s'_0)$ as a function of $s'_0$.
4. For the noise estimate $n''_j$ the derivation is very similar. However, we first have to calculate the distribution $f_A(a_j)$ for the average $a_j$ of two magnitude values $m_j$.

5. Then the distribution $f_{S''}(s'')$ for the signal-to-noise ratio $s'' = \frac{m_j}{n''_j}$ is calculated.
6. Finally, $f_{S''}(s'')$ is integrated to estimate the probability for a specific signal-to-noise ratio $s''_0$ to be reached by chance which gives the significance $p_{S''}(s''_0)$ as a function of $s''_0$.

*Distribution $f_M(m_j)$ of one spectral magnitude $m_j$*

We assume the following 2D-Gaussian distribution $f_N$ for the real part $x$ and the imaginary part $y$ of a Fourier component

$$f_N(x, y) = \frac{1}{2\pi\sigma^2}\, e^{-\frac{x^2+y^2}{2\sigma^2}} \tag{22}$$

$f_N$ has a rotation-invariant symmetry, as $f_N$ depends on the magnitude $\sqrt{x^2 + y^2}$ irrespective of the phase. Thus Equation (22) can be simplified by polar coordinates. The following equations describe the transition from $x$ and $y$ to the polar coordinates magnitude $(m_j)$ and phase $(\varphi_j)$, where the magnitude is given in multiples of $\sigma$

$$x = \sigma m_j \cos(\varphi_j) \tag{23a}$$

$$y = \sigma m_j \sin(\varphi_j) \tag{23b}$$

Substituting $m_j$ and $\varphi_j$ in Equation (22) yields

$$f_N(m_j, \varphi_j) = \frac{m_j\sigma^2}{2\pi\sigma^2}\, e^{-\frac{(\sigma\cdot m_j)^2}{2\sigma^2}} \tag{24}$$

$$f_N(m_j, \varphi_j) = \frac{1}{2\pi} m_j\, e^{-\frac{{m_j}^2}{2}} \tag{25}$$

where the following Jacobi matrix was used for coordinate transformation

$$\begin{vmatrix} \frac{\partial x}{\partial m_j} & \frac{\partial y}{\partial m_j} \\ \frac{\partial x}{\partial \varphi_j} & \frac{\partial y}{\partial \varphi_j} \end{vmatrix} = \begin{vmatrix} \sigma\cos(\varphi_j) & \sigma\sin(\varphi_j) \\ -\sigma m_j \sin(\varphi_j) & \sigma m_j \cos(\varphi_j) \end{vmatrix} = m_j\sigma^2 \tag{26}$$

As Equation (25) does not depend on $\varphi_j$, the probability distribution $f_M(m_j)$

for the magnitude $m_j$ of a pure noise response is

$$f_M(m_j) = \int_0^{2\pi} f_N(m_j, \varphi_j)\, \mathrm{d}\varphi_j = m_j\, e^{-\frac{{m_j}^2}{2}} \int_0^{2\pi} \frac{1}{2\pi}\, \mathrm{d}\varphi_j = m_j\, e^{-\frac{{m_j}^2}{2}} \tag{27}$$

which is the *Rayleigh* distribution [12].

*Noise estimate by a control response*

Here the signal-to-noise ratio s′ is defined by the ratio of two magnitude values $m_j$. A specific value for $s'$ can be achieved in different ways, e.g., when the magnitude of the control condition is $m_j$, then the signal magnitude has to be $s' \cdot m_j$. Thus $f_{S'}(s')$ is the joined probability of $f_M(m_j)$ and $f_M(s' \cdot m_j)$, integrated over the entire magnitude range for $m_j$

$$f_{S'}(s') = \int_0^{\infty} m_j\, f_M(m_j)\, f_M(s' \cdot m_j)\, \mathrm{d}m_j \tag{28}$$

$$f_{S'}(s') = \int_0^{\infty} m_j \cdot m_j\, e^{-\frac{{m_j}^2}{2}} \cdot (s' \cdot m_j)\, e^{-\frac{(s' \cdot m_j)^2}{2}}\, \mathrm{d}m_j \tag{29}$$

$$f_{S'}(s') = \frac{2s'}{(1 + s'^2)^2} \tag{30}$$

There is one final step in calculating the statistical significance $p_{S'}(s'_0)$. The probability $f_{S'}(s')$ is integrated for all situations in which a specific signal-to-noise level $s'_0$ is reached or exceeded

$$p_{S'}(s'_0) = \int_{s'_0}^{\infty} f_{S'}(s')\, \mathrm{d}s' \tag{31}$$

$$p_{S'}(s'_0) = \frac{1}{1 + {s'_0}^2} \tag{32}$$

This is the desired function for the statistical significance of any signal-to-noise ratio $s'_0$, where $s'_0$ is defined as the ratio of one spectral line to the corresponding spectral line of a control condition at the same spectral line. Figure 3e illustrates the shape of $p_{S'}(s'_0)$ and some examples are given in Table 1.

*Noise estimate by averaging the two neighboring magnitude values*

Now we will derive the probability distribution $f_A(a_j)$ for the average $a_j$ of two magnitude values. The evaluation of the probability distribution $f_{S''}(s'')$ for $s''$ is similar to the derivation of Equation (32). A certain average magnitude $a_j$ can be realized in many ways, e.g., when the magnitude at the frequency to the left is $m_{j-1}$, the magnitude at the frequency to the right has to be $m_{j+1} = 2a_j - m_{j-1}$. As magnitude values are always positive, an appropriate magnitude $m_{j+1}$ can only be found for $0 \leq m_{j-1} \leq 2a_j$. Thus $f_A(a_j)$ is the joined probability of $f_M(m_{j-1})$ and $f_M(2a_j - m_{j-1})$, integrated over an appropriate magnitude range for $m_{j-1}$

$$f_A(a_j) = 2 \int_0^{2a_j} f_M(m_{j-1}) f_M(2a_j - m_{j-1}) \, \mathrm{d}m_{j-1} \tag{33}$$

$$f_A(a_j) = 2 \int_0^{2a_j} m_{j-1} \, e^{-\frac{{m_{j-1}}^2}{2}} (2a_j - m_{j-1}) \, e^{-\frac{(2a_j - m_{j-1})^2}{2}} \, \mathrm{d}m_{j-1} \tag{34}$$

$$f_A(a_j) = e^{-2a_j^2} \left( 2a_j + e^{a_j^2} (2a_j^2 - 1) \sqrt{\pi} G(a_j) \right) \tag{35}$$

where $G$ denotes the Gaussian error function

$$G(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} \mathrm{d}t \tag{36}$$

A certain value for the signal-to-noise ratio $s''$ can be realized in different ways, e.g., when the average magnitude of the two neighboring frequencies is $a_j$, then the signal magnitude has to be $s'' \cdot a_j$. Thus $f_{S''}(s'')$ is the joined probability of $f_A(a_j)$ and $f_M(s'' \cdot a_j)$, integrated over the entire magnitude range for $a_j$,

$$f_{S''}(s'') = \int_0^{\infty} a_j f_A(a_j) f_M(s'' \cdot a_j) \, \mathrm{d}a_j \tag{37}$$

$$f_{S''}(s'') = \int_0^\infty a_j\ e^{-2a_j^2} \left(2a_j + e^{a_j^2}(2a_j^2 - 1)\sqrt{\pi}\,G(a_j)\right)$$
$$(s'' \cdot a_j)\ e^{-\frac{(s''\cdot a_j)^2}{2}} \mathrm{d}a_j \qquad (38)$$

$$f_{S''}(s'') = -\frac{2s''\left(s''^4\sqrt{\frac{1}{2+s''^2}} - 2s''^2\left(2\sqrt{\frac{1}{2+s''^2}} + \sqrt{2+s''^2}\right)\right)}{(2+s''^2)^{\frac{3}{2}}(4+s''^2)^2}$$
$$+\frac{8s''\left(6\sqrt{\frac{1}{2+s''^2}} + \sqrt{2+s''^2}\right)}{(2+s''^2)^{\frac{3}{2}}(4+s''^2)^2}$$
$$-\frac{s''\sqrt{2}\left(-4+s''^2\right)\arctan(\sqrt{\frac{2}{2+s''^2}})}{(2+s''^2)^{\frac{5}{2}}} \qquad (39)$$

Similar to the derivation of Equation (32), there is one final step in evaluating the statistical significance $p_{S''}(s''_0)$. $f_{S''}(s'')$ has to be integrated for all situations in which a certain signal-to-noise level $s''_0$ is reached or exceeded.

$$p_{S''}(s''_0) = \int_{s''_0}^\infty f_{S''}(s'')\ \mathrm{d}s'' \qquad (40)$$

$$p_{S''}(s''_0) = \frac{2 + 4\frac{1}{2+s_0''^2}}{4+s_0''^2} - \frac{\sqrt{2}s_0''^2\arctan(\sqrt{\frac{2}{2+s_0''^2}})}{\left(2+s_0''^2\right)^{\frac{3}{2}}} \qquad (41)$$

This is the desired function for the statistical significance of any signal-to-noise ratio $s''_0$, where $s''_0$ is defined as the ratio of one spectral line over the average of its two neighbors. Figure 4e illustrates the shape of $p_{S''}(s''_0)$ and some examples are given in Table 2.

## Appendix II

In this section we present two tables that show how the two formulas (12b/32) and (18b/41) can be implemented in an Excel spreadsheet. The numbers and formulas are inserted into the first column of an empty Excel spreadsheet. Thus the cell names are Al, A2, … We use *text in italics* to describe a

*Table 3.* Noise estimated by a control response, Excel notation. The Fourier components of the signal and noise response are inserted into the first two cells Al and A2. Cell A3 contains the formula for the signal-to-noise ratio $s_0'$. Cell A4 gives the significance level $p_{S'}(s_0')$ as evaluated by Equation (12b/32)

| Cell name | Cell content |
|---|---|
| A1 | *signal magnitude* |
| A2 | *noise magnitude* |
| A3 | **= A1/A2** |
| A4 | **=1/(1+A3ˆ2)** |

*Table 4.* Noise estimated by averaging two spectral neighbors, Excel notation. The signal magnitude and the noise magnitudes of the two neighboring frequencies are inserted into the cells Al, A2, and A3. Cell A4 contains the formula for the signal-to-noise ratio $s_0''$. Cell A5 is used to simplify the formula in A6, which gives the significance level $p_{S''}(s_0'')$ as evaluated by Equation (18b/41)

| Cell name | Cell content |
|---|---|
| Al | *noise magnitude at the first neighbor* |
| A2 | *signal magnitude* |
| A3 | *noise magnitude at the second neighbor* |
| A4 | **=A2/ ((A1+A3) /2)** |
| A5 | **=2+A4ˆ2** |
| A6 | **=(2+4*(1/A5))/(4+A4ˆ2)** |
| | **– (SQRT(2)*A4ˆ2*ARCTAN(SQRT(2/A5)))/A5ˆ(3/2)** |

specific magnitude value that is inserted in the corresponding cell. **Text in boldface** shows a formula in the Excel notation that relates the output of the corresponding cell to the content of other cells.

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