As for absolute accuracy, it is well known that the statistical average of the square of the output of a filter of bandwidth B_n, measured for a time interval, T, will have a mean square fractional error given by $(B_n T)^{-1}$ to within factors the order of unity (Rice, 1954). For fixed observation time, say some fraction of the time over which necrotizing processes in a biological preparation noticeably change the preparation, for greatest accuracy one must use the greatest filter bandwidth. The bandwidth must also be compatible with its usefulness in allowing one to identify the spectral density from the observations. We have shown that an octave filter allows one to identify the spectral density. Hence any narrower filter will yield less accuracy. This conclusion flatly contradicts the intuitive idea that one often encounters, and which DeFelice and Sokol assume, namely, that to achieve accuracy one must use filters with bandwidths much less than their center frequency.

DeFelice and Sokol determine the mean square deviation of their integral filter and compare it, in their Eq. 40, with the mean square error, $(B_n T)^{-1}$, of a general band-pass filter. They give the ratio as $2\pi l/B$ for measuring frequency independent noise, and as $2\pi l/5B$ for measuring noise varying as f^{-2}. For an octave filter at frequency $(f_1 f_2)^{1/2} = l$, $B = l/(2)^{1/2}$. Hence their result states that the filter error is $[2\pi(2)^{1/2}]^{1/2}$, and $[2(2)^{1/2}\pi/5]^{1/2}$ times that of their integral spectrum. These are factors the order of unity, and are hardly a firm basis for choosing either method for observing noise in biological membranes.

Received for publication 7 September 1976 and in revised form 29 November 1976.

REFERENCES

DEFELICE, L. J., and B. A. SOKOL. 1976. A new analysis for membrane noise. *Biophys. J.* **16**:827.

HALFORD, D. 1968. A general mathematical model for $|f|^{\alpha}$ spectral density with special reference to flicker noise $1/|f|$. *Proc. Inst. Electr. Electron. Eng.* **56**:251.

ITT ENGINEERS. 1968. Reference data for radio engineers. 5th edition. Howard W. Sams & Co., Inc., Publishers, Indianapolis. 8-1 to 8-4.

RICE, S. O. 1954. Mathematical analysis of random noise. *In* Noise and Stochastic Processes, N. Wax, editor. Dover Publications, Inc., New York. Sec. 3.9 and Appendix 4*c*.

STRANDBERG, M. W. P., and E. I. HAMMER. 1975. Current-fluctuation noise in toad urinary bladder during active transport of sodium ions. *J. Appl. Phys.* **46**:3661.

M. W. P. STRANDBERG
Department of Physics and Research Laboratory of Electronics
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Comments on the Analysis of Membrane Noise by the "Integral Spectrum" Procedure

Dear Sir:

Membrane noise reflects events on a microscopic scale and its characterization has the potential of discriminating between various models of ionic conduction. Its study includes three distinct phases: (*a*) acquisition of reliable data, (*b*) analysis of the resulting random process, and (*c*) testing of hypotheses and model fitting. The limited time, T, over which the preparation may yield stationary data and the mixture of several components originating from separate processes are some of the difficulties encountered.

In the context of (*b*), DeFelice and Sokol (1976) have recently discussed a procedure called the "integral spectrum" (I) in comparison with classical power density spectrum (S) analysis. They assert that "it is now evident that power spectral density estimates are not sufficiently accurate to distinguish between available models" and claim that "one advantage of the new method is the accuracy of the experimental curve ... since the statistical error in I is greatly improved over that of S from the same length of data". Unfortunately, the initial statement is not further substantiated. And the claim that the I procedure can possibly yield a more reliable data base for model fitting is not valid and arises from an incomplete comparison between S and I.

Given a record of length T, S analysis yields spectral estimates with a normalized variance $\sigma^2 = 1/B_S T$, where B_S is the analysis bandwidth, $1/B_S < T$. The estimates are consistent, and when B_S is narrow compared to spectral features of significance, they are also unbiased. A bank of N such filters, selected so they are adjacent and essentially not overlapping, e.g. such as are readily synthesized by discrete Fourier transform, yield an efficient estimate of the power density spectrum. The data available for model fitting consists of N discrete points, each with normalized σ^2 as above. It is important to note that these points are statistically independent (at least for a Gaussian process, e.g. see Bendat and Piersol, 1971) hence they provide nonredundant information.

In contrast, in the I analysis, *a*) the effective noise bandwidth of analysis, B_I, is purposely chosen to be much larger than B_S, (*b*) successive filters overlap considerably, and (*c*) B_I is not constant, but rather increases linearly with frequency.

Condition *c* constitutes prewhitening for a $1/f$ process. This does not modify the intrinsic statistical uncertainty of the data, but can only reduce additional errors during processing as a consequence of limited dynamic range of instruments. Certainly dynamic range is not an acute problem for present membrane data. Condition *c* is not related to meaningful considerations of accuracy but rather to matters of subjective preference, i.e. of a $1/f$ process showing as an horizontal rather than -1 slope line on log-log coordinates.

Conditions *a* and *b* are significant. Since the filters used for I analysis have wider bandwidth, their mean square outputs have smaller σ^2, of course. But it is important to note that the number of data points is correspondingly reduced. Furthermore, since the filter responses overlap very significantly, these fewer data points are not statistically independent and supply partially redundant information. For the filters selected by DeFelice and Sokol, all the data points have some degree of mutual correlation and adjacent points are very strongly correlated. They briefly mention the reduced frequency resolution of I analysis. However, they do not explicitly consider the corollary reduction in the number of data points, nor their correlation. Rather, the claim of improved statistics for I is based solely on the standard deviations of single data points. I analysis does give plots that appear quite "smooth" but it must be realized that this smoothness, in large part, results precisely from the large correlation between adjacent points, and does not necessarily reflect a genuine improvement in statistical confidence.

In fact, a simple heuristic argument readily shows that S contains more information than I (in the sense of information theory) and that to be otherwise would contradict basic notions of signal analysis. Given an S analysis, the corresponding I spectrum can be derived exactly. The converse is not true. This follows since the mean square output from any I filter can be obtained as a weighted sum of the mean square outputs of the collection of the S filters in the same pass-band. This sum can be computed exactly, hence without information loss. The I spectrum, then, cannot contain more information than its source, the S spectrum. Given I, the corresponding S cannot be reconstructed exactly, in spite of the statement by DeFelice and Sokol that "curve-fitting could be used to regain the original information." Once energies from different (orthogonal, independent) frequency components have been lumped into a single number (such as an I data point), the original components cannot be retrieved unless other information is available. Clearly, then, the data provided by S for subsequent model fitting is

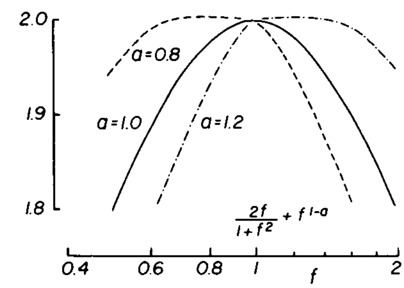

FIGURE 1 Plot of integral spectrum computed for the sum of Lorentzian and $1/f^a$ components. Both components have unity power spectral density at the relaxation frequency (also normalized to unity) and a ranges between 0.8 and 1.2. These conditions are representative of actual membrane noise data. The peaking frequency of the integral spectrum may differ significantly from the relaxation frequency if $a \neq 1$. Other conditions and/or additional components may result in further bias or even in a nonpeaking spectrum.

an upper bound for that of I and the claim that more accurate data can be extracted through an I procedure cannot hold.

In addition to this conceptual discussion, the following points appear appropriate: Whether an S or an I data base is used, only detailed curve-fitting can, in final analysis, extract parameters of model assumptions. For instance, the fact that for a simple relaxation process over a $1/f$ background the I spectrum peaks at the relaxation frequency is indeed convenient. However there is no a priori assurance that the peak of any particular record corresponds to that frequency until analysis of the entire spectrum supports the assumption of a precisely $1/f$ background. While conduction in the voltage-dependent potassium channels shows an important component that, generically, can be referred to as "$1/f$", individual records show that a more accurate description is of the form $1/f^a$, with a range between 0.8 and 1.2, and mean value of 1 (Poussart, 1971, Fishman, et al., 1975). As shown in Fig. 1, such a range of a may produce large biases in the peaking frequency of the I spectrum. White and rising high frequency noise from instrumentation can further bias the peaking frequency. In other words the peak of the I spectrum cannot be simply equated with a natural mode without detailed curve-fitting.

While the narrow resolution of S analysis is not theoretically needed for resolving the broad features of membrane noise, it offers an important practical advantage. Obvious wild points, such as components clearly related to power line leakage, can be edited out with confidence (e.g. see the 60-Hz component edited in Fig. 3*a* of DeFelice and Sokol for white plus $1/f$ simulation). In I analysis the corresponding artifact is mixed with the data (e.g. see corresponding bump in Fig. 4*b*, which would not be immediately distinguishable from a relaxation component) and cannot be removed unless, of course, other information such as provided by S indicates that the feature arises from an obvious extraneous source. The resolution of S facilitates removal of such components before curve-fitting and hypothesis testing.

In conclusion, the critical comments expressed here are not that I analysis is an inappropriate procedure but rather that there is no theoretical basis to its claim of improved intrinsic accuracy. The use of filters with constant relative bandwidth is hardly new. For instance, "third-octave" filter banks are commercially available and have been commonly used in accoustics. Even for membrane noise, the first voltage noise (Derksen and Verveen, 1966) and current noise (Poussart, 1969) data were processed in this manner, but only because means for real-time digital computation of S were not readily available at the time.

Received for publication 1 October 1976.

REFERENCES

BENDAT, J. S., and A. G. PIERSOL. 1971. Random Data: Analysis and Measurement Procedures. Wiley-Interscience, John Wiley & Sons, Inc., New York.

DEFELICE, L. J., and B. A. SOKOL. 1976. A new analysis for membrane noise: The integral spectrum. *Biophys. J.* **16**:827.

DERKSEN, H. E., and A. A. VERVEEN. 1966. Fluctuations of resting neural membrane. *Science (Wash. D.C.)* **151**:1388.

FISHMAN, H. M., L. E. MOORE, and D. J. M. POUSSART. 1975. Potassium-ion conduction noise in squid axon membrane. *J. Membrane Biol.* **24**:305.

POUSSART, D. J. M. 1969. Nerve membrane current noise: direct measurements under voltage clamp. *Proc. Nat. Acad. Sci. U.S.A.* **64**:95.

POUSSART, D. J. M. 1971. Membrane current noise in lobster axon under voltage-clamp. *Biophys. J.* **11**: 211.

D. POUSSART
Laboratoire de Bionique
Département de Génie Électrique
Université Laval
Québec, Canada