Fluctuation Analysis of Nonideal Shot Noise

Application to the Neuromuscular Junction

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ABSTRACT Procedures are described for analyzing shot noise and determin-

ing the waveform, $w(t)$, mean amplitude, $\langle h \rangle$, and mean rate of occurrence, $\langle r \rangle$

and by measuring the spontaneous rate of occurrence of miniature endplate

potentials (MEPPs) or currents. Continuous measurements of the MEPP rate and

amplitude during prolonged periods of intense secretion provide data that can or corrected for before λ_3 and λ_2 can be used to calculate $\langle r \rangle$ and h. This article

derives the frequency composition of λ_2 (power spectrum) and λ_3 (skew "bispec-

phenomenon on these spectra can be virtually eliminated by appropriately integration: the integrals with $m \neq -n$ equal zero, whereas those with $m = -n$

equal T. Therefore,

record extends over the interval $[t = 0, T]$, then only those events occurring between the times $[-\tau]$ and $[T]$ will contribute to it. If K is the number of such events, then $V(t)$ is given by:

$$V(t) = \sum_{j=1}^{K} hw(t - \theta_j), \tag{5}$$

where j is an arbitrary event index (not implying time sequence), and θ_j is a random variable, representing the time of occurrence of the j^{th} event.

If the process is Poissonian, the individual events occur independently and the probability density functions of the θ_j's are all equal. Let $p(t)dt$ be the probability for each of the K events to occur in the infinitesimal time interval between t and $t + dt$. If an ensemble of records is available, each with the same time course for $p(t)$, then the expected value of $V(t)$ for the ensemble is:

$$E[V(t)] = E\left[\sum_{j=1}^{K} hw(t - \theta_j)\right] = hE[K]E[w(t - \theta_j)] = hE[K]\int_{-\tau}^{T} p(t')w(t - t')dt'.$$

Since $E[K]p(t')$ is the expected rate of occurrence of events, $r(t')$, at time t', we have:

$$E[V(t)] = h\int_{-\tau}^{T} r(t')w(t - t')dt' = h\int_{0}^{\infty} r(t - u)w(u)du, \tag{6}$$

where $u = t - t'$, and the change in the limits of integration is justified because

$$E[\tilde{v}(n)] = \int_0^T \left[h \int_0^\infty r(t-u)w(u)du \right] dt \; e^{-2\pi int/T}$$

$$= h \int_0^\infty w(u)du \; e^{-2\pi inu/T} \int_{-u}^{T-u} r(t')dt \; e^{-2\pi int'/T} \qquad (8)$$

$$\{t' = t - u, \; n \neq 0\}.$$

Neglecting errors at the edges and changing the limits of integration of t' to [0,

where $\tilde{w}(n)$ and $\tilde{r}(n)$ are the finite Fourier transforms of $w(t)$ and $r(t)$, respectively

If the events occur independently, then the distribution of the K's is Poissonian so that $E[K(K-1)] = (E[K])^2$. Since $E[K]p(t') = r(t')$, then $E[K(K-1)]p(t')\cdot p(s') = r(t')r(s')$, and we have:

$$E[V(t)V(s)] = h^2 \int_{-\tau}^{T} r(t')w(t-t')w(s-t')dt' + h^2 \int_{-\tau}^{T} r(t')w(t-t')dt' \int_{-\tau}^{T} r(s')w(s-s')ds' \quad (10)$$

$$= h^2 \int_{-\infty}^{\infty} r(t')w(t-t')w(s-t')dt' + E[V(t)]\,E[V(s)]$$

where the limits of the first integral have been extended to $\pm\infty$, since the integrands are zero for $t' < -\tau$ or $t' > T$.

If Eq. 10 is substituted into Eq. 9, we get:

$$E[\tilde{v}_2(n)] = \int_0^T dt \int_0^T ds\, E[V(t)V(s)]\, e^{2\pi in(t-s)/T}$$

If we define the power densities of $v(t)$, $r(t)$, and $w(t)$, respectively, as:

$$G_v(n) = 2\tilde{v}_2(n)/T,$$

$$G_r(n) = 2\tilde{r}_2(n)/T,$$

and

$$G_w(n) = 2\tilde{w}_2(n),$$

then we have:

$$E[\langle v^2\rangle] = \frac{1}{T}\sum_{n=1}^{\infty} E[G_v(n)] = \frac{1}{T}\sum_{n=1}^{\infty} h^2 G_w(n)[G_r(n)/2 + \langle r\rangle]. \qquad (13a)$$

Frequency composition of the skew of shot noise. The expected value of the skew can be computed in a similar way from the expected value of $\tilde{v}_3(n, m)$:

$$E[\tilde{v}_3(n, m)] = \int dt \int ds \int dz\ E[V(t)V(s)V(z)]\ e^{2\pi i(nt+ms-nz-mz)/T}$$

give, respectively: $h^3\langle r\rangle T\tilde{w}_3(n, m)$, $h^3\tilde{r}_2(n + m)\tilde{w}_3(n, m)$, $h^3\tilde{r}_2(m)\tilde{w}_3(n, m)$, $h^3\tilde{r}_2(n)\tilde{w}_3(n,$

$m)$, and $h^3\tilde{r}_3(n, m)\tilde{w}_3(n, m)$. Thus, we obtain:

$$E[\tilde{v}_3(n, m)] \sim h^3\tilde{w}_3(n, m)\ [\langle r\rangle T + \tilde{r}_2(n) + \tilde{r}_2(m) + \tilde{r}_2(n + m) + \tilde{r}_3(n, m)].$$

The expected value of the skew is (from Eq. 4, with n, m, $n + m \neq 0$):

show that these extra components are additive and appear only within the frequency bandwidth of $r(t)$. They can, in principle, be filtered out whenever the bandwidth of $r(t)$ is narrower than that of $w(t)$, and $\langle r \rangle$ can then be computed from the semi-invariants of the filtered signal. The filtering is practical only when a portion of the spectrum of $w(t)$ can be unambiguously identified in the noise spectrum. The parameters of $w(t)$ are then deduced by fitting the analytical function for its power spectrum to this region of the noise spectrum, and the

Effects of a Nonuniform Distribution of Shot Amplitudes

When the shot events are not uniform in amplitude, then the equation for the semi-invariants of the fluctuations is (Rice, 1944):

$$\lambda_n = \langle r\rangle\langle h^n\rangle I_n = \langle r\rangle\langle h\rangle^n D_n I_n, \qquad (18)$$

where $D_n = \langle h^n\rangle/\langle h\rangle^n$ is a factor that depends upon only the distribution of h. If we use $\langle h\rangle$ and $\langle r\rangle$ to denote the apparent mean amplitude and mean rate of the events as determined from the skew and variance, and use $\langle h\rangle_t$ and $\langle r\rangle_t$ to denote "true" average values, we get:

$$\langle h\rangle = (\lambda_3/I_3)/(\lambda_2/I_2) = \langle h^3\rangle/\langle h^2\rangle = \langle h\rangle_t D_3/D_2.$$

$$\langle r\rangle = (\lambda_2/I_2)^3/(\lambda_3/I_3)^2 = \langle r\rangle\langle h^2\rangle^3/\langle h^3\rangle^2 = \langle r\rangle_t D_2{}^3/D_3{}^2.$$

Thus, $\langle r\rangle$ and $\langle h\rangle$ will be in error whenever h is not uniform. If the distribution of the h's is known, from a histogram, for example, then D_n can be calculated

Once γ is known, then the D_n's, R, and the correction factors for $\langle r \rangle$ and $\langle h \rangle$ [illegible]

can be calculated:

$$D_n = (\gamma + n)!/(\gamma + 1)^n \gamma!;$$

$$R = \langle h^3 \rangle^2/\langle h^4 \rangle \langle h^2 \rangle = D_3{}^2/D_4 D_2 = (\gamma + 3)/(\gamma + 4);$$

$$\langle r \rangle_t/\langle r \rangle = (\gamma + 3)^2/(\gamma + 2)(\gamma + 1) = R^2/(3R - 2)(2R - 1);$$

$$\langle h \rangle_t/\langle h \rangle = (\gamma + 1)/(\gamma + 3) = (3R - 2)/R.$$

[illegible]

Conversely, γ and the correction factors can be determined from the values of [illegible] conductance are too small to change the time constant significantly (see below).

We assume that conductances sum linearly and that the added endplate con-

an average rate, $\langle r \rangle$, such that the mean conductance of the endplate is increased 50% above its resting value. If MEPPs summed linearly, the average membrane where $V_d = V_E - V_R$ is the mean driving potential. If MEPPs summed linearly

around point B, the displacement, v_l, of the potential from V_R caused by a small

$$v_l = \alpha_B V_d \delta g / G_B. \tag{22}$$

The actual displacement, v_m, is obtained by solving Eq. 20 when $V = V_R + v_m$ and

$\alpha = \alpha_B$:

$$v_m/(1 - v_m/V_d) = \alpha_B V_d \delta g / G_B, \tag{23}$$

which becomes, according to Eq. 22:

$$v_m/(1 - v_m/V_d) = v_l. \tag{24}$$

This is the original Martin correction. It can be used because the time-dependent properties of the equivalent circuit remain at their average values and are not

Since the semi-invariants are linearly related to $\langle r \rangle$ (Rice, 1944), the second terms in the last two equations are of the order of $\langle r \rangle^2$. These terms are

responsible for most of the error, becoming significant only when $\langle r \rangle$ is large

(>200/s; Fesce et al., 1986, Fig. 4). The term involving λ_4 in the third equation

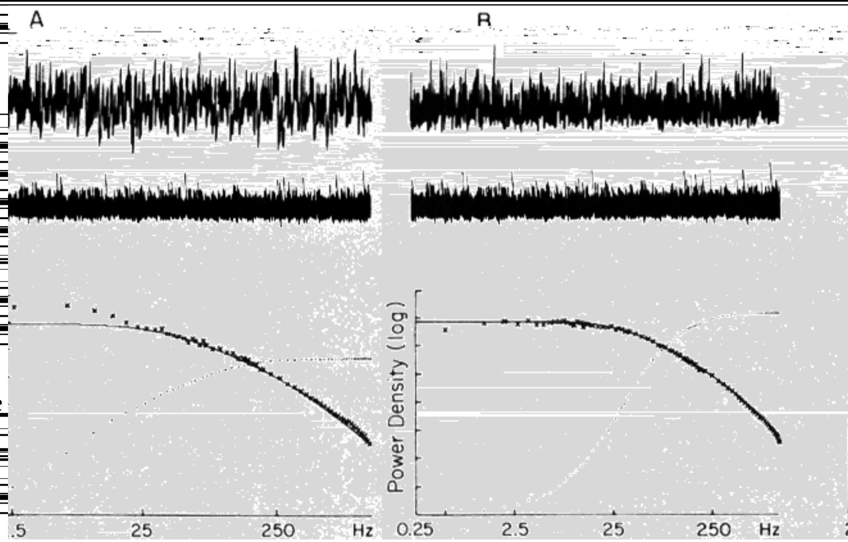
Eq. 27 shows that λ_4 of an arbitrary distribution measures the departure of u_4

from that of a Gaussian. Since the distribution of shot noise approaches a Gaussian

r rises. If we assume for the moment that $\langle r \rangle$ is stationary and the shots are

uniform, then we expect: $u_4 = \langle r \rangle h^4 I_4 + 3(\langle r \rangle h^2 I_2)^2$. As $\langle r \rangle$ rises, λ_4 becomes a changing in a random stepwise manner to stimulate random volleys of MEPPs. The first

was summed with the output of a pseudo-white-noise generator (model 132, Wavetek, independently simulated records where successive points were 2 ms apart (instead of the



Five [...] rate (~1,000/s), were simulated using superimposed volleys of artificial MEPPs. The following parameters were used: (*a*) 5 volleys/s; a mean volley duration of 0.6 s;

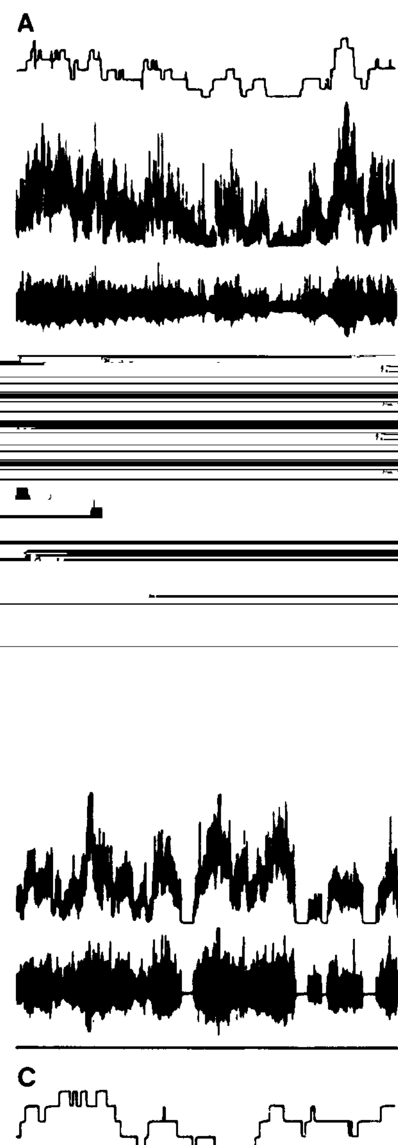
FIGURE 2. (*A*) Simulated fluctuations in potential at an endplate. The MEPP rate is stationary (500/s) and the MEPP time constants are 5.0 and 0.5 ms. Top trace: unfiltered record, 10 s duration; middle trace: the same record filtered through a 1-ms high-pass RC filter. The vertical calibration is arbitrary, and the filtered record is displayed at a higher gain. Lower panel: power spectrum of the fluctuations

of 500/s per volley; steps smoothed by low-pass filtering (RC = 4 ms): baseline rate, 50/s.

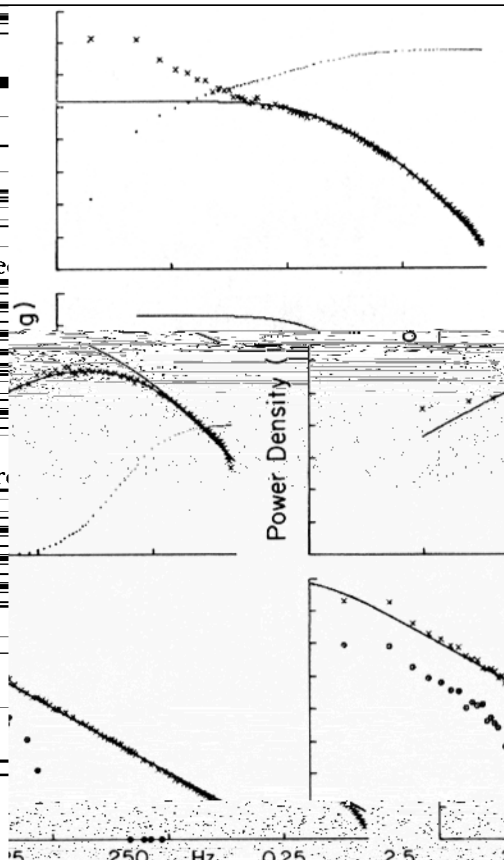
The top traces of Fig. 4, *A–C*, show examples of the time courses of $r(t)$ obtained [redacted] using the parameters listed above (*a–c*). These stepwise-changing rates were used to generate the records shown in the middle trace of the panels; the lowest set of traces shows the records after filtering (RC = 1 ms). Since the mean

Fig. 6 shows the bias and random errors obtained when the $\langle r \rangle$ and h o f the artificial MEPPs were estimated from records like those in Fig. 4. The bias errors

were 50–100% for estimates made from un filtered records and the random



errors were large. Using the mean and variance, ⟨r⟩ was underestimated, while



h was overestimate[…] n the variance and

skew of filtered r[…] errors than those

computed from the mean and variance, but all errors decreased as the filter RC decreased: when RC = 1 ms. the random errors were reduced to ~10% (compare

Similar results were obtained when $r(t)$ was varied in a continuous random

manner. The fractional errors (mean ± SD) in the estimates obtained from mean

A

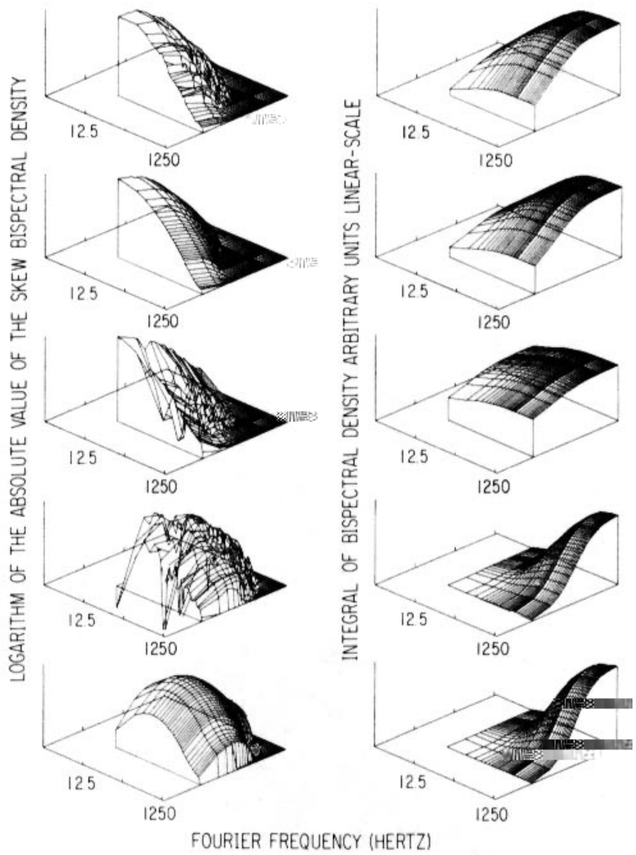


FIGURE 7. (Left) Bispectral density of the skew. To obtain this plot, a record was

(RC = 1 ms). The random errors in the estimates of $\langle r \rangle$ and h were reduced further when the moments of three independent filtered records were averaged. This averaging procedure is equivalent to increasing by threefold the duration of a data-collection interval, and the resultant reduction in the random errors agrees with the predictions of the theory (Appendix).

The skew bispectra of the simulated records shown in Fig. 7 support the theoretical prediction that the contributions of nonstationarities to the skew are

We also simulated stationary ($\langle r \rangle = 032/s$) records of filtered MEPPs whose h

factors were distributed in accordance with γ distributions. In one set of simu-

lations, the β parameter of the distributions was fixed at 1, while γ varied from noise and the filtered waveform, $w'(t)$, are identical and are equally affected by further changes in the filter time constant. Therefore, consistency among the

estimates of $\langle r \rangle$ and h obtained with different filters is strong empirical evidence

Effects of Shot Nonuniformity and Nonlinear Summation

in two additional ways: the individual shots may not be identical in amplitude or waveform, and they may not add linearly. When the shots are not equal in

amplitude, bias errors arise because $\langle h^n \rangle$ is greater than $\langle h \rangle^n$ and their ratio less rapidly than an exponential, i.e., the absolute value of $[dr(t)/dt]/r(t)$ decreases as t increases; (c) $r(t)$ changes exponentially with time. We assume that the duration of one

event, τ, is much shorter than the duration of the record, T (see Theory). In the first two the skew are less than $(1 + \epsilon)^{3/2} - 1 \sim (3/2)\epsilon$; the normalized bias error for the skew computed in the filtered signal will also be less than $(3/2)\epsilon$ (in our example, <15%).

Random Errors Involved in the Various Procedures

In the Theory section, we have taken the expectations over ensembles of records with the same $r(t)$ and T. When, in experimental work, only one such record is available, the expected standard errors in the estimates must be known in order to assess the reliability of the results obtained from it.

The normalized standard error of the mean is given by:

Since $E[\langle v^2\rangle]^2 = \sum_{x=-\infty}^{\infty} \sum_{y=-\infty}^{\infty} h^4\tilde{w}_2(x)\tilde{w}_2(y)[\tilde{r}_2(x) + \langle r\rangle T][\tilde{r}_2(y) + \langle r\rangle T]/T^4$, the expected square error of the variance is:

$$\sum_{x=-\infty}^{\infty} \sum_{y=-\infty}^{\infty} h^4\tilde{w}_2(x)\tilde{w}_2(y) \frac{2\mathrm{Re}[\tilde{r}_3(x,y)+\tilde{r}_3(x,-y)]+2[\tilde{r}_2(x)+\tilde{r}_2(y)+\tilde{r}_2(x+y)]+\langle r\rangle T}{T^4}. \quad \text{(A4)}$$

When this is normalized to the square value of the variance of a stationary record [given [illegible]

by the double sum in x and y of $h^4\tilde{w}_2(x)\tilde{w}_2(y)(\langle r\rangle/T)^2$], we have a standard square error of [illegible]

t [illegible] e variance:

$$(\sigma_2)^2 \leqslant \mathrm{Max}\left[\frac{2\mathrm{Re}[\tilde{r}_3(x, y) + \tilde{r}_3(x, -y)] + 2[\tilde{r}_2(x) + \tilde{r}_2(y) + \tilde{r}_2(x + y)]}{(\langle r\rangle T)^2}\right] + \frac{1}{\langle r\rangle T}. \quad \text{(A5)}$$

It is apparent that all the factors produced by nonstationarity (those within the brackets) [illegible]

Integrating over t and neglecting edge errors:

$$\frac{1}{T}\int_0^T dt \sum_{j=1}^{K} \int_0^T [h_j w_j(t-t')]^n p_j(t')dt' = \frac{1}{T}\sum_{j=1}^{K}(h_j)^n \int_0^T p_j(t')dt' \int_0^\infty [w_j(t)]^n dt$$

$$= \frac{1}{T}\sum^{K}(h_j)^n \int^\infty [w_j(t)]^n dt = \frac{K}{T}\frac{1}{K}\sum^{K}(h_j)^n (I_n)_j$$

Therefore, even when the events are inhomogeneous and their occurrences are non-random, non-Poissonian, or correlated, a factor $\langle r\rangle \overline{h^n I_n}$ is present in the n^{th} moment

Fesce, R. 1986. Fluctuation analysis applied to non-stationary biological processes. *In* Calcium, Neuronal Function and Transmitter Release. R. Rahamimoff and B. Katz, editors. Martinus

Fesce, R., J. R. Segal, B. Ceccarelli, and W. P. Hurlbut. 1986. Effects of black widow spider venom and Ca^{2+} on quantal secretion at the frog neuromuscular junction. *Journal of General Physiology*. 88:59–81.

Finger, W., and H. Stettmeier. 1981. Analysis of miniature spontaneous inhibitory postsynaptic currents (sIPSCs) from current noise in crayfish opener muscle. *Pflügers Archiv European*