NEURONAL EXOCYTOSIS EXHIBITS FRACTAL BEHAVIOR

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ABSTRACT

The time sequence of exocytic events in both neurons and non-neuronal cells exhibits fractal (self-similar) properties, as evidenced by a number of statistical measures. Such fractal activity occurs in neurotransmitter secretion at Xenopus neuromuscular junctions and rat hippocampal synapses in culture, and in the exocytosis of exogenously supplied neurotransmitter from cultured Xenopus myocytes and rat fibroblasts. The magnitude of the fluctuations of the rate of exocytic events about the mean decreases slowly as the rate is computed over longer and longer time periods, the periodogram decreases in power-law fashion with frequency, and the Allan factor (relative variance of the number of exocytic events) increases as a power-law function of the counting time. These features are hallmarks of self-similar behavior. Their

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description requires models that exhibit long-range, power-law-decaying correlation (memory) in event occurrences. We have developed a physiologically plausible model that accords with all of the statistical measures that we have examined: the fractal lognormal-noise-driven doubly stochastic Poisson process (FLNDP). In particular, we show that the experimental rate function is well modeled by fractal lognormal noise (FLN). The appearance of behavior with fractal characteristics at synapses, as well as in systems comprising collections of synapses, indicates that such behavior is an inherent property of neuronal signaling.

INTRODUCTION

Communication in the nervous system is mediated by action-potential-initiated exocytosis of multiple vesicular packets (quanta) of neurotransmitter (Katz, 1966). Even in the absence of such action potentials, however, many neurons spontaneously release individual packets of neurotransmitter (Fatt and Katz, 1952). On arrival at the postsynaptic membrane, the neurotransmitter (ACh) molecules induce elementary endplate currents (EECs), which take the form of nonstationary two- (or multi-) state on-off sequences (Sakmann, 1992). Current flows when the ACh channel is open (i.e., when its two binding sites are occupied by agonist), and ceases when the channel is closed. A postsynaptic miniature endplate current (MEPC) comprises some 1000 EECs (Sakmann, 1992). It was shown by Del Castillo and Katz (1954) that superpositions of MEPC-like events comprise the postsynaptic endplate currents elicited by nerve impulses.

It has generally been assumed that the sequence of MEPCs forms a memoryless stochastic process (Fatt and Katz, 1952). However, Rotshenker and Rahamimoff (1970) discovered that exocytosis in the frog neuromuscular junction can exhibit correlation (memory) over a period of seconds, provided that extracellular Ca^{2+} levels are elevated above their normal values.

We have studied the statistical properties of exocytic events over a far larger range of time scales than previously examined (Lowen et al, 1997). MEPCs were recorded from innervated myocytes in Xenopus nerve-muscle cocultures and from rat hippocampal neurons in cell culture. MEPCs from non-neuronal preparations were also examined: the quantal secretion of ACh from isolated myocytes (autoreception) and from rat fibroblasts, both exogenously loaded with ACh (Dan and Poo, 1992; Girod et al., 1995).

We have directed our attention toward those statistical measures that reveal the presence of memory. Our analysis reveals that the time sequences of the MEPCs, and therefore of the underlying exocytic events, exhibit memory that decays away slowly, as a power-law function of time, in both neuronal and non-neuronal cells. This long-duration correlation is present over the entire range of time scales investigated, which stretches to thousands of seconds. The occurrence of a MEPC therefore makes it more likely that another MEPC will occur at some time thereafter. The analysis of long MEPC data sets reveals that the rate of events is consistent with a fractal process, exhibiting fluctuations over multiple time scales. Fractals are objects which possess a form of self-similarity: parts of the whole can be made to fit to the whole by shifting and stretching. The hallmark of fractal behavior is power-law dependence in one or more statistical measures, over a substantial range of the time (or frequency) scales at which the measurement is conducted (Lowen and Teich, 1995).

STATISTICAL MEASURES

Perhaps the simplest measure of a sequence of neuronal activity is its rate: the number of events registered per unit time. For vesicular release events, even this straightforward measure has fractal properties; the fluctuations of the rate do not decrease appreciably even when a very long counting time is used to compute it. This behavior derives from correlations in the sequence of interevent intervals, as confirmed by the observation that the fractal properties of the rate estimate are destroyed by shuffling (randomly reordering) the intervals. This operation removes the correlations among the intervals while exactly preserving their relative frequencies.

Another measure sensitive to fractal behavior is the Allan factor (AF) (Teich et al., 1996), defined as the ratio of the Allan variance of the event count to the mean. The Allan variance, in turn, represents the average variation in the difference between adjacent counts (Allan, 1966). To compute the Allan factor at a specified counting time T, the data record is first divided into adjacent counting windows of duration T, and the number of events falling within each window recorded. The difference between the counts in each window and the following window is then computed; the mean square of this quantity forms the Allan variance. Dividing this quantity by twice the average number of counts in each window yields the Allan factor. In general the Allan factor A varies with the counting time T. For fractal point processes, including all exocytosis recordings of sufficient length that we have analyzed, A rises as a power-law function of T for large counting times T (Lowen et al, 1997). Again, shuffling the intervals destroys this effect, rendering the Allan factor essentially constant with counting time.

The periodogram (PG), an estimator of the power spectral density, also reveals the presence of fractal activity. Much as for continuous-time processes, the power spectral density reveals how power is concentrated in various frequency bands. For low frequencies f (corresponding to long time scales T), the PG also varies in a fractional power-law fashion with frequency for the same data recordings, although with a negative exponent (Lowen et al, 1997). Finally, the PG computed from a shuffled version of the vesicular activity, in contrast, does not vary in power-law fashion, lending further credence to the notion that it is the ordering of the intervals, rather than their relative sizes, which is particularly responsible for the fractal aspects of the vesicular activity.

MODEL

A model of exocytic activity which successfully fits both the apparent fractal behavior seen over long time scales and interevent statistics such as the interevent-interval histogram (IIH) is the fractal-lognormal-noise driven Poisson point process (FLNDP), which we develop as follows.

Verveen and Derksen (1968) showed that the voltage of an excitable-tissue membrane at rest exhibits fractal ($1/f$-type) fluctuations with a Gaussian amplitude distribution, which they traced to fluctuating K^+-ion concentrations. Voltage-gated Ca^{2+}-ion channel openings are responsible for vesicular exocytosis (Zucker, 1993). For a fixed membrane voltage near the resting potential, calcium flow is negligible. Occasionally, however, random thermally induced channel openings will occur, often leading to spontaneous exocytic events for nearby vesicles. Such spontaneous behavior is almost completely memoryless, and is therefore well modeled by a homogeneous Poisson process (HPP) (Cox and Lewis, 1966), with a fixed rate λ given by the Arrhenius equation (Berry et al., 1980): $\lambda = \mathcal{A}\exp(-E_A/\mathcal{R}\mathcal{T})$. Here the rate

λ is the number of vesicular release events expected to occur in a unit time interval, $\mathcal{A}$ is a rate constant, E_A is the constant activation energy associated with the ion-channel opening, $\mathcal{R}$ is the thermodynamic gas constant, and $\mathcal{T}$ is the absolute temperature. (That only a fixed proportion of the channel-opening events leads to exocytosis can be incorporated into the value of $\mathcal{A}$.) Living cells generate non-zero voltages across their membranes which modify the rate, leading to $\lambda = \mathcal{A}\exp[-(E_A - zFV)/\mathcal{R}\mathcal{T}]$, with z the valence of the charge involved in the channel opening, F the Faraday constant (coulombs/mole), and V the membrane voltage. Different fixed membrane voltages lead to spontaneous exocytic patterns which differ only in their average rates; all are HPPs. These rates are exponential functions of the membrane voltage, as prescribed by this equation.

However, the membrane voltage V is not fixed, but rather varies randomly in time, exhibiting Gaussian fluctuations which appear fractal; it is therefore well modeled by fractal Gaussian noise, or FGN [or a modified form of fractal Brownian motion, depending on the exponent α_S of the $1/f^{\alpha_S}$ noise $V(t)$]. Thus the rate λ of the Poisson process will also vary in time. Since the rate is the exponential transform of the voltage, which is described by FGN, the rate will behave as fractal lognormal noise (FLN). The resulting openings are therefore characterized by a *doubly stochastic* (rather than homogeneous) Poisson process with a rate that is FLN. Finally, then, the calcium-flow events, and therefore the exocytic events, are described by the FLNDP model. Unlike a homogeneous Poisson process, the FLNDP is not memoryless. Rather, the fluctuating membrane voltage imparts fractal correlations to the exocytic events so that the observation of a short (long) interevent time, for example, signifies a locally high (low) rate $\lambda(t)$, which in turn indicates that the next interevent time is also likely to be short (long).

Analytical predictions and computer simulations based on this three-parameter model were compared with the exocytic-event data for a variety of statistical measures. In particular, agreement with the data was excellent over all time scales for the AF, PG, and IIH (Lowen et al, 1997), with the same simulations employed for all three measures. Moreover, the AF and PG calculated from shuffled FLNDP simulations are in excellent accord with those of the shuffled data. We conclude that, aside from its physiological plausibility, the FLNDP provides an excellent mathematical model for characterizing the sequences of MEPCs observed in our experiments (Lowen et al, 1997).

LOGNORMAL RATE

In addition to serving as an excellent model for the the *point process* of exocytic events, the FLNDP process developed above also models the *rate* $\lambda(t)$ of event generation, which we now proceed to examine. Estimates of the rate were collected from a representative neuromuscular junction used for collecting AF and PG statistics (Lowen et al, 1997) by counting the number of exocytic events occurring in windows of $T = 1$, 3, 10, 30, 100, and 300 seconds. Each count was then divided by the window duration, to obtain a rate estimate, which was then expressed as a survivor function. The rate was relatively constant even over the $T = 300$ sec window, so that our estimate is indeed of the rate. Some fluctuation does exist, however, which serves to negatively bias the variance of the rate estimate for these longer windows. In contrast, the shorter counting times suffer from a lack of resolution, since the counting process necessarily yields an integral number of counts; this count is often zero for the shortest counting times. We plot rate estimates obtained for a range of counting times in Fig. 1 (solid curves), where the lowest curves on the left correspond to the shortest counting

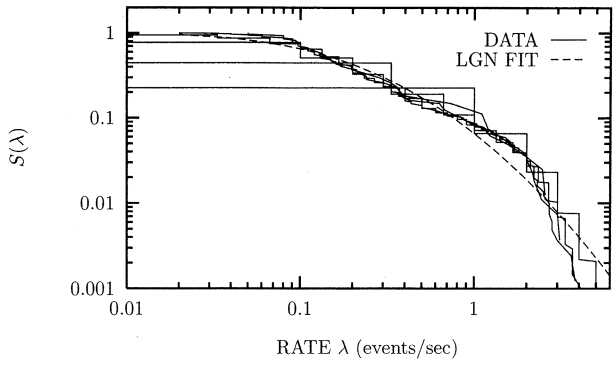

Figure 1. Doubly logarithmic plot of the estimated survivor function $S(\lambda)$ of the rate vs. the rate λ (probability that the rate is larger than the abscissa value) for spontaneous vesicular release obtained from a Xenopus neuromuscular junction (solid curves). The sequence of events from which this plot has been constructed has a duration $L = 8164$ sec and contains $N = 2644$ interevent intervals. Counting times were $T = 1$, 3, 10, 30, 100, and 300 seconds, increasing from the bottom up at the left edge of the figure. Also shown is the theoretical lognormal fit (dashed curve), which agrees with the experimental rate functions.

times. These rate estimates compare favorably with the theoretical lognormal rate function (dashed curve).

It will be of interest to conduct measurements of both the presynaptic membrane voltage and the exocytic process, for which the FLNDP model makes the following predictions. Given the parameters of $V(t)$, the rate $\lambda(t)$ can be shown to have a mean, variance, and autocorrelation function given by

$$\begin{aligned} \mathrm{E}[\lambda] &= \mathcal{A}\exp(\mu+\sigma^2/2) \\ \mathrm{Var}[\lambda] &= \mathcal{A}^2\exp(2\mu+2\sigma^2) \\ R_\lambda(\tau) &= \mathrm{E}^2[\lambda(t)]\exp\left\{(zF/\mathcal{RT})^2\left[R_V(\tau)-\mathrm{E}^2[V]\right]\right\}, \end{aligned} \tag{1}$$

respectively, where we have defined

$$\begin{aligned} \mu &= (E_A - zF\mathrm{E}[V])/\mathcal{RT} \\ \sigma &= zF\sqrt{\mathrm{Var}[V]}/\mathcal{RT}, \end{aligned} \tag{2}$$

and $R_V(\tau)$ is the autocorrelation function of the membrane voltage $V(t)$. Furthermore, if the rate $\lambda(t)$ [or equivalently the voltage $V(t)$] exhibits fluctuations which are slow in comparison with the average rate of channel openings $\mathrm{E}[\lambda]$, then the moments and probability density function for the times t between openings are, respectively, given by

$$\mathrm{E}[t^n] = n!\mathcal{A}^{-n}\exp\left[-n\mu+(n^2-2n)\sigma^2/2\right] \tag{3}$$

$$\begin{aligned} p(t) &= \pi^{-1/2}\mathcal{A}\exp\left(\mu+3\sigma^2/2\right) \\ &\quad\times\int_{-\infty}^{\infty}\exp\left[-x^2-t\exp\left(\mu+2\sigma^2+\sqrt{2}\sigma x\right)\right]dx. \end{aligned} \tag{4}$$

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