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Fractal behavior of spontaneous neurotransmitter release: from single-synapse to whole-cell recordings

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***Abstract*—Spontaneous release of neurotransmitter vesicles at brain chemical synapses has been deeply investigated in the last decades at several levels. First and second order statistics have been widely adopted as a tool for assessing, inter-alia, dependence of spontaneous release on the concentration of ionic species in the intra- or extra-cellular environment. Furthermore, several studies demonstrated that spontaneous release exhibits fractal, and generally non purely random, behavior. Most experimental work on this topic exploits population whole-cell patch-clamp recordings in order to acquire post-synaptic currents elicited by neurotransmitter release into the synaptic cleft. Since several synapses merge on the dendritic arbor of a single neuronal cell, whole-cell recordings of miniature excitatory post-synaptic currents (mEPSCs) implies the temporal superimposition of releasing events from all active synapses on the arbor. This limitation can be overcome by exploiting the loose-patch clamp technique on single synapses, thus acquiring spontaneous release events from individual synapses. Here, we present results obtained by applying well-established methods for the quantification of fractal behavior in the series of mEPSCs acquired through the use of both whole-cell and single-synapse loose-patch recording techniques on hippocampal neurons and synapses. Our long-term aim is to get a better understanding of the release process and of the mechanisms of neuronal integration when the information is coming from several simultaneously active synaptic sites.**

I. Introduction

Several experimental and computational studies demonstrated that the electrical activity of neuronal units both *in vitro* and *in vivo* (besides *in silico*) is able to produce interesting fractal behaviors [1-4]. Many hypothesis about the origin of such behaviors can be found in the literature, in most cases supported by computational modeling of neuronal units or groups. In order to obtain a better comprehension of these phenomena, we are interested into the non-linear features of spontaneous neurotransmitter release, also known as quantal releases due to spontaneous fusion of synaptic vesicles at the synaptic active zone. Spontaneous release of quanta is accepted to be a probabilistic process where each synaptic vesicle or quantum is discharged randomly and independently one from another at individual release sites. The process underlying quanta generation is usally considered a random Poisson process which should produce an exponential distribution of intervals between successive quanta (inter-event intervals, IEIs). The histograms of inter-event intervals (IIH) could also be fitted by the more general gamma distribution [5]. Previous studies revealed a divergence of the spontaneous release from a purely Poisson process for CNS synapses and that the alternation of high frequency bursts of releases and almost silent periods causes the IIH to be best-fitted by a sum of exponentials [6,7]. In literature, there are no clear evidences available in favor of power-laws (i.e. scaling behavior, a typical feature of fractal signals) in the distribution of these IEIs. On the contrary, it has been pointed out by Lowen et al. [8] that the rate of the releasing process, at different levels of the nervous system, does exhibit fractal behavior. Starting from these results, the model proposed by the authors for describing quantal release is the fractal-rate Poisson point process, i.e. a non-homogeneous Poisson process (NHPP), driven by a stochastic-fractal rate. Based on biological considerations, it has been suggested that the underlying rate process is likely to be a fractal lognormal noise (FLN), and this model was actually used to fit the available data. It is worth noting that measurements of spontaneous release events used in this study were population measurements, therefore they cannot reflect release activity from single units.

Here, we provide examples of the application of fractal analysis methods to both whole-cell and single-synapse recordings of miniature release events recorded in the presence of tetrodotoxin (TTx). These recordings were analyzed in order to evaluate the consistency of signal generation models proposed by others for describing the case of single-synapse recordings and the consistency with population data.

II. Materials and Methods

A. Hippocampal cell cultures

Postnatal CA3-CA1 hippocampal cultures were prepared from postnatal day 4 and 5 neonatal rats as described in [9]. Neurons were used for single-synapse recordings after 10-21 days in vitro.

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B. Electrophysiology

Whole-cell (WC) recordings were obtained at the soma of neurons by standard or perforated WC patch clamp (electrode resistance 2–5MΩ). For synaptic loose-patch (LP) recordings, synapses were labeled with FM1–43 and electrically isolated by the recording pipette (tip diameter 2μm, pipette resistance 0.5–2MΩ, seal resistance 2–11MΩ). Current traces were filtered at 2–5 kHz and stored using a digital tape recorder. mEPSCs were recorded in the presence of 0.5-1 μM TTx. For a detailed description of all protocols and preparations as well as for data preprocessing please refer to [7,9]. All the analysis presented here have been performed on the sequences of mEPSCs occurrences that have been extracted from the recordings in LP and WC configurations. The set of data used here for the analysis consists of 3 recordings in LP configuration and 3 recordings in WC configuration. Average duration of WC recordings is 2898 s, with an average of 8929 events, while the average duration of LP recordings is 413 s with an average of 271 events. Potential issues about differences in duration and number of events will be treated in the next paragraphs.

C. Probability Distributions

Cumulative probability distributions of the IEIs are computed and fitted by gamma distributions in order to access the Poisson nature of the point process. The gamma distribution provides good fit of IIHs for WC recordings, while IIHs of LP recordings are affected by the presence of a limited number of out-layer intervals, which indicate the alternation of burst periods to silent periods. If the out-layers are discarded, the IIH is well fitted by the gamma distribution and previous studies prove that a sum of exponentials can also well fit these histograms. Since we are interested in higher order statistics (long-term correlations and fractal properties), these results are sufficient to assume an exponential-like distribution of the intervals and to discard interval-based measures for more suited rate-based measures.

D. Quantification of the Fractal Behavior

Several analytical methods have been provided by previous studies for the detection of fractal behavior for a variety of signal classes. A widespread feature of long-term correlated time series is a 1/f power-law in the low-frequency range of the power spectra, which is closely related to the self-similar (i.e. fractal) properties of the time series. As for the fractal-based point processes, this quantification approach can also be applied. An exhaustive coverage of this topic is given by [10]. As the authors suggest, the most suitable blind method (i.e. without *a priori* knowledge about the process generation model) for quantifying the fractal exponent α in a point process is by fitting the 1/f spectrum of the underlying rate process in the low-frequency range. Starting from simulation studies, the authors infer that this measure, besides being the most accurate, is the most reliable since the 1/f rate spectrum is present for all classes of fractal-based point processes. The algorithm that we exploited is based on the description given by [10] and is the following: i) estimation of the process rate: a counting window of length T = 0.01s is moved among the entire length of the point process, events are counted and the result is divided by T, providing a constant-sampling estimate of the instantaneous rate with sufficient time resolution; ii) computation of the periodogram of the rate estimate; iii) power spectral components are averaged in the frequency domain; iv) linear fitting of the periodogram slope in the low-frequency range, on a double logarithmic scale. The absolute value of the linear slope represents an estimation of the fractal exponent α.

E. Surrogate Data

In order to discard any non-linear correlation in the point process, the position of IEIs of the original sequence is randomized according to an uniform distribution. This procedure does not alter the IIH. A set of 20 shuffled series is obtained and represents the surrogate set for a single recording. For each surrogate point process, rate periodogram is computed according to the previously described algorithm. Then, an average periodogram is computed and fitted for the estimation of the surrogate data fractal expontent α_{surr}. This exponent is likely to be 0 if the point process is a purely fractal-rate point process.

III. Results

As an example, in Fig. 1 are shown two traces of detected mEPSC occurrences from population (above, WC) and single-synapse (below, LP) recordings. As previously described [6-7,9], WC and LP recordings are different, the latter showing fewer events, given comparable time horizons and, more remarkably, LP having events grouped into bursts, separated by rather long silent periods. The bursting behavior clearly affects standard statistical description of the spontaneous release process, as previously reported [6-7,9]. Recording durations were different for technical reasons, but a truncation of WC recordings to get higher comparability of data would not be a smart choice since longer series duration means lower frequency spectral components in the periodogram and thus a more reliable estimation of α.

Results from the application of the estimation algorithm are shown in Fig. 2 and Fig. 3 for the same recordings just presented. In both figures, the black line represents the rate spectrum estimation (periodogram) for the original point process, while the blue one represents the mean rate periodogram computed on the related surrogate data set. All periodograms are shown in a doubly logarithmic scale, in order to make more clear the linear slope of the spectral components in the low-frequency range.

For both WC and LP recordings, the change in the slope from $1/f^{\alpha}$ to flat spectrum is rather evident and fitting was easily achieved. A difference in the low-frequency slope is also evident comparing the two recordings. WC recordings exhibit a higher α value. A clear difference between the two results is given by comparing the spectra of the surrogate data sets.

For WC recordings, the surrogate data spectrum is clearly flat: it is likely that all the fractal features of WC signals resides in the underlying rate process only, and that the α estimation is reliable.

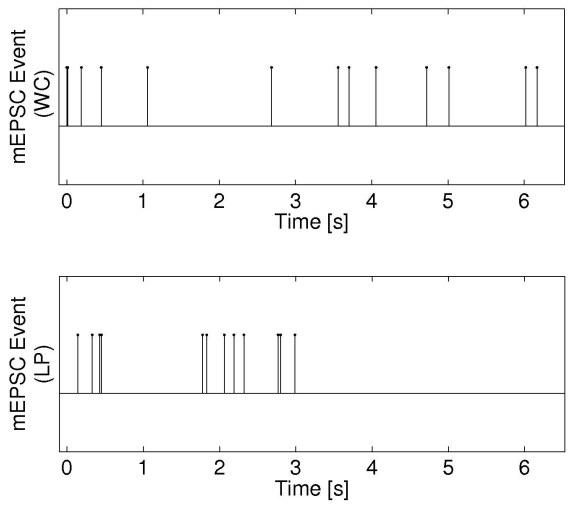

Fig. 1. The temporal sequence of mEPSC events recorded whit whole-cell configuration (WC) and single-synapse loose-patch (LP).

This is not inferable from the LP spectrum. Indeed, even if a 1/f behavior is rather evident and quantifiable, the same shuffling process does not totally flatten the spectrum in the low frequency range.

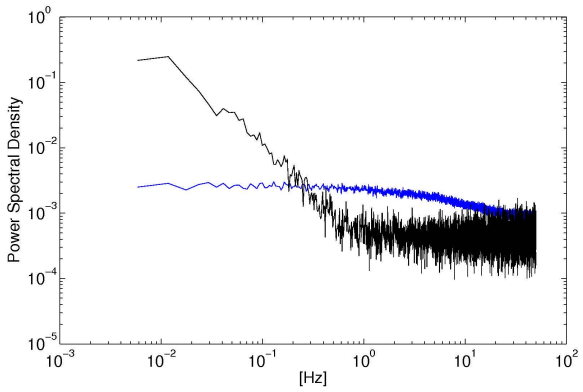

Fig. 2. Estimation of α for the WC recording. Black line: periodogram of the estimated rate process (α = 1.439); blue line: average periodogram computed on the surrogate data set (α_{surr} = 0.022).

A summary of the results for the all data set is given by Tab. 1, where average values of the estimated α for both LP and WC recordings, as well as standard deviations, are provided.

A t-test was performed between LP and WC sets of α values, which resulted to be significantly different in the two recording configurations ($p<0.05$).

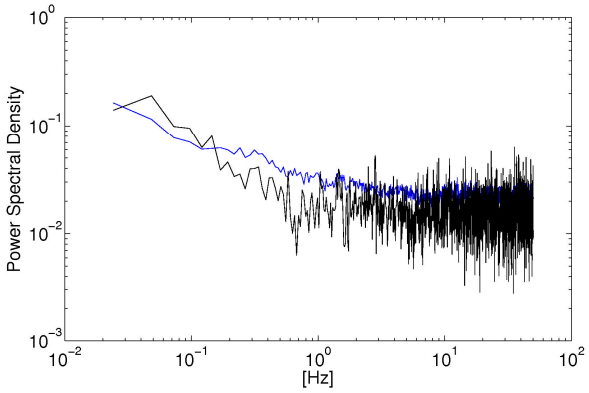

Fig. 3. Estimation of α for the LP recording. Black line: periodogram of the estimated rate process (α = 0.729); blue line: average periodogram computed on the surrogate data set (α_{surr} = 0.379).

	α_{mean}	α_{std}	$\alpha_{mean,surr}$	$\alpha_{std,surr}$
WC	1.300	0.172	0.050	0.028
LP	0.681	0.043	0.405	0.025

Tab. 1: Average values and standard deviation of estimated α for WC and LP recordings and related surrogate data sets.

IV. Discussion

The presented results come from the application of well-known analytical methods for the quantification of fractal behavior of point processes to a data set with small sample numerosity. In our view the most interesting aspect of this work is represented by the comparison between single-synapse (LP) and population (WC) recordings.

WC recordings, by placing the recording electrode at the cell soma, are summing the activity of a large number of synapses located far away from recording sites and produce events highly affected by post-synaptic cable attenuation, summation and filtering. Moreover, in the WC configuration many events might not be detected or detected individually. Missing data reduce the representativeness of the sample from each synapse and alter the statistical behavior of release events. On the contrary, as previously described, single-synapse data are definitely closer to the source of the phenomenon. At least in theory, in a neuronal cell with a short and well clamp post-synaptic dendritic tree, WC occurrences should result from merging several release sequences coming from those synapses which are contacting the post-synaptic neuronal arbor.

A complete knowledge about the mechanisms underlying this merging process is not available, and a detailed comparative analysis of results from single-synapse and population recordings might shed some light on a process which is not as trivial as one would imagine based on cable

theory.

As shown in this paper by using the surrogate data test, single synapse recordings (LP) are not fitted to the model of pure fractal-rate Poisson point process, which we have hypothesized starting from the population case-study in [8].

Starting from these preliminary results, it is possible to infer that the pure fractal-rate Poisson point process is not well suited to describe the occurrence of spontaneous quanta at individual hippocampal synapses. A trivial summation of individual synaptic release sequences, given the bursting feature of LP recordings, could explain the more homogeneously distributed WC process. Nevertheless, such a trivial superimposition of the single synapse release events should not affect the fractal exponent value, which, on the contrary, appears to be, on average, significantly higher with population somatic recordings.

Starting from these considerations, we might infer that the merging process mediated by the dendritic arbor actuate not only a spatio-temporal summation, but also an integration of the original series (increase in the exponent generally means integration).

V. Conclusions

This work is aimed at presenting some preliminary results of the application of well-established methods for fractal behavior assessment to a set of spontaneous neurotransmitter release events series. The innovation is represented by the single-synapse loose patch recordings, which have been compared with the more standard whole-cell recordings.

The hope is to extend soon this comparison to larger experimental data sets in order to provide some deeper insights on the pre- and post-synaptic mechanism underlying the generation and/or modulation of fractal behavior of miniature events.

References

[1] C.C. King, "Fractal and chaotic dynamics in nervous systems.," Progress in neurobiology, vol. 36, Jan. 1991, pp. 279-308.

[2] V. Matveev and X.J. Wang, "Differential short-term synaptic plasticity and transmission of complex spike trains: to depress or to facilitate?," Cerebral cortex (New York, N.Y. : 1991), vol. 10, Nov. 2000, pp. 1143-53.

[3] M.C. Teich, C. Heneghan, S.B. Lowen, T. Ozaki, and E. Kaplan, "Fractal character of the neural spike train in the visual system of the cat," Journal of the Optical Society of America A, vol. 14, Mar. 1997, p. 529.

[4] P.G. Aitken, T. Sauer, and S.J. Schiff, "Looking for chaos in brain slices.," Journal of neuroscience methods, vol. 59, Jun. 1995, pp. 41-8.

[5] R.N. Leao, F.N. Leao, and B. Walmsley, "Non-random nature of spontaneous mIPSCs in mouse auditory brainstem neurons revealed by recurrence quantification analysis.," Proceedings. Biological sciences / The Royal Society, vol. 272, Dec. 2005, pp. 2551-9.

[6] A. Abenavoli, L. Forti, and A. Malgaroli, "Mechanisms of spontaneous miniature activity at CA3-CA1 synapses: evidence for a divergence from a random Poisson process," The Biological Bulletin, vol. 199, 2000, p. 184.

[7] A. Abenavoli, L. Forti, M. Bossi, A. Bergamaschi, A. Villa, and A. Malgaroli, "Multimodal quantal release at individual hippocampal synapses: evidence for no lateral inhibition.," The Journal of neuroscience : the official journal of the Society for Neuroscience, vol. 22, Aug. 2002, pp. 6336-46.

[8] S.B. Lowen, S.S. Cash, M.-ming Poo, and M.C. Teich, "Quantal Neurotransmitter Secretion Rate Exhibits Fractal Behavior," J. Neurosci., vol. 17, 1997, pp. 5666-5677.

[9] L. Forti, M. Bossi, a Bergamaschi, a Villa, and a Malgaroli, "Loose-patch recordings of single quanta at individual hippocampal synapses.," Nature, vol. 388, Aug. 1997, pp. 874-8.

[10] S.B. Lowen and M.C. Teich, Fractal-based point processes, John Wiley and Sons, 2005.