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Fractal-like correlations of the fluctuating inter-spike membrane potential of a *Helix aspersa* pacemaker neuron

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ABSTRACT

We analyzed the voltage fluctuations of the membrane potential manifested along the inter-spike segment of a pacemaker neuron. Time series of intracellular inter-spike voltage fluctuations were obtained in the current-clamp configuration from the F1 neuron of 12 *Helix aspersa* specimens. To assess the dynamic or stochastic nature of the voltage fluctuations these series were analyzed by Detrended Fluctuation Analysis (DFA), providing the scaling exponent α. The median α result obtained for the inter-spike segments was 0.971 ([0.963, 0.995] lower and upper quartiles). Our results indicate a critical-like dynamic behavior in the inter-spike membrane potential that, far from being random, shows long-term correlations probably linked to the dynamics of the mechanisms involved in the regulation of the membrane potential, thereby endorsing the occurrence of critical-like phenomena at a single-neuron level.

1. Introduction

In recent decades, several efforts in the study of the dynamics of neural activity seeking to understand the interaction between the environment and the internal control mechanisms of neurons have been performed. A fractal-like behavior ($1/f$) in the temporal fluctuations for the occurrence of action potentials, or inter-spike time intervals, collected from extracellular recordings of spontaneous electrical activity of different types of neurons has been found; identifying, as well, that this behavior is linked to the dynamic frequency of depolarization [1–4], neural facilitation or plasticity, and signal transmission [5,6]. Also, a complex behavior of the membrane voltage fluctuations in nerve fibers has been detected and these fluctuations have been studied to assess encoded information by long-term memory processes [7,8].

Early studies suggested that intracellular recordings of the membrane voltage fluctuations in nerve fibers can be related to thermal movements generated by the flow of potassium and sodium ions through the plasma membrane [9,10]. Contrastingly, other experiments indicated that these fluctuations are rather due to structural changes in the voltage-dependent potassium channels [11–13]. Furthermore, other authors have shown theoretically and experimentally that ionic channels show dynamic internal movements and continuous changes of conformational states with long-term memory [14–18]. Besides, the inter-spike membrane potential of neurons is modified by the activation of different mechanisms such as the synaptic transmission, the activation of ionic channels, and the modulation of cytoplasmic free calcium concentration $[Ca^{2+}]_i$, which all modulate the frequency of occurrence of action potentials [19,20]. Thus, we hypothesized that the intracellular voltage fluctuations of the inter-spike segment present non-random dynamics. Accordingly, in this research we analyzed the intracellular voltage fluctuations of the membrane potential manifested along the inter-spike segment of the *Helix aspersa* F1 pacemaker neuron, which is involved in the innervation of sensory organs like the osfradio [21]. Our results here indicate a critical-like dynamic behavior that, far from being random, shows fractal-like correlations probably as an expression of the dynamics of the mechanisms involved in regulating the membrane potential.

2. Materials and methods

2.1. Biological preparation

The subesophageal ganglion cell identified as F1 of the *H. aspersa* specimen was used in this study owing to its depolarization pattern,

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topographical location and straightforward manipulation [22,23]. Some of the main mechanisms that generate its electrical activity are well known as it becomes the case for the calcium-dependent potassium channels (BK, SK), voltage-dependent potassium channels (Kv), sodium and chlorine channels (Na^+, Cl^-) [24–30], voltage-gated calcium of L-type channels, HCN4 channels, and G protein-activated inwardly-rectifying potassium channels [31]. Furthermore, within the main mechanisms considered to involve in establishing the inter-spike membrane potential are SK channels, HCN4 channels, and G protein-activated inwardly-rectifying potassium channels [32–34]. In addition, the largest neurons of *H. aspersa* such as the F1 cell are known to present 8 synaptic inputs [59].

The ganglia used were exposed to type XIV protease (Sigma-Aldrich) in a concentration of 5 mg/10 ml for 8 min. Afterwards the action of the enzyme was stopped by washing the preparation with standard ringer solution and the connective tissue sheath that covers the neurons was removed by mechanical microdissection.

2.2. Ringer solution and electrodes

Borosilicate based glass capillaries were used once stretched with a DKI 700-C puller device (2 mm diameter wall with filament). The electrodes were filled with 3 M KCl solution giving a resistance of 5–10 MΩ. The ganglia were perfused with the Ringer solution with the following composition: NaCl 75 mM, KCl 4 mM, $CaCl_2$ 10 mM, $MgCl_2$ 5 mM, Hepes 5 mM, and pH 7.5 was adjusted with NaOH to a concentration 1 M. All salts were provided by J.T. Baker brand.

2.3. Electrophysiological recordings

Intracellular recordings were performed in the current-clamp configuration using a Dagan 8500 amplifier, and the voltage tracings were monitored on a Tektronik oscilloscope. For the acquisition and visualization of the data a Digidata 1200 A/D interface (Axon Instruments) was employed. A sampling frequency of 1 kHz was used, and the signal to noise ratio (SNR) was estimated at 27.7 dB. This measure was obtained by dividing the root-mean-square (RMS) values of a typical inter-spike segment and the noise generated from the setup (Fig. 1).

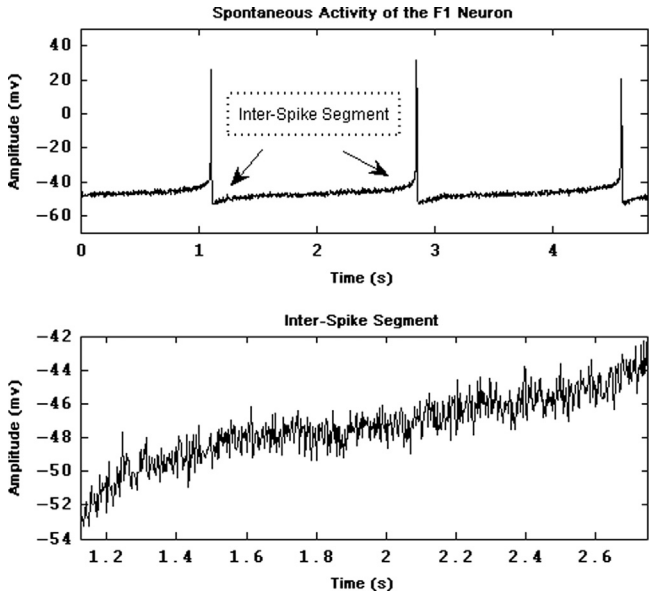

Fig. 1. Typical voltage fluctuations of the membrane potential manifested along the inter-spike segment of the F1 neuron which presents pacemaker type activity (27.7 dB SNR was estimated).

To characterize and compare the noise generated by our setup with the dynamics of the inter-spike segment, 10 data series containing an average of 340 ± 10 samples before (control) and after introducing the electrode into the F1 neuron of one specimen were obtained (Fig. 2, left).

To further study the voltage fluctuations in the membrane potential of the inter-spike segment, 12 additional specimens were used, and for each F1 neuron of these specimens all segments (from 16 to 20) containing an average of 340 ± 10 samples were considered (Fig. 3, left). The suitability of these numbers of samples is in accordance with previous studies estimating the scaling exponent from short segments of synthetic fractal-like data [35].

2.4. Inter-spike voltage fluctuations series and Detrended Fluctuation Analysis (DFA)

The inter-spike segments were then analyzed with DFA as described below to obtain the scaling exponent α. About 18 scaling exponents per neuron were obtained, which were averaged to obtain a representative scaling exponent of the dynamics present in the voltage fluctuations of the inter-spike segment. This procedure was performed for each of the 12 specimens studied.

Numerical control groups involving segments with approximately 340 samples were also constructed to compare the dynamics presented in the inter-spike segments; these groups were obtained using either random permutations of the same inter-spike segments or synthetic fractal-like data ($1/f$) generated by the spectral synthesis approximation [36]. The Kruskal–Wallis test was used for the statistical comparisons because the scaling exponents α obtained from the analysis of voltage fluctuations of the inter-spike segments did not present a normal distribution (significance level $P < 0.05$).

The Detrended Fluctuation Analysis (DFA) has proven useful to explore long-range correlations in time series [37]. This method provides a quantitative parameter called the scaling exponent α that is estimated as follows.

The original voltage fluctuations time series is numerically integrated and its mean subtracted.

$$y(k) = \sum_{i=1}^{k} x(i) - \bar{x}$$

The resulting time series $y(k)$ is divided in nonoverlapping boxes of equal length n and for each box or scale a least-square fit is used to obtain $y_n(k)$. The local linear trend of $y(k)$ is removed by substracting $y_n(k)$ from each box and to compute the root mean square fluctuation $F(n)$ as

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[y(k) - y_n(k)]^2}$$

This computation is repeated over different n values to provide a relationship between $F(n)$ as a function of box size or time scale n.

$$F(n) \approx n^{\alpha}$$

A linear relationship is considered by applying a logarithmic operator on both sides of the previous equation; thereby, the fluctuations can be characterized by estimating the slope or scaling exponent α (within the scales $n=4$ to $n=N/4$) [37]. Roughly, if $\alpha = 0.5$, the series has random fluctuations; if $\alpha \approx 1$, the series presents long-term or fractal-like correlations.

3. Results

3.1. Inter-spike data and experimental control groups

The median α results obtained with DFA for the voltage fluctuations of the noise before the electrode which were introduced