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Time-scales in the interplay between calcium and voltage dynamics

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Abstract

The interaction between slow calcium dynamics and voltage dynamics increases the processing capabilities of neurons. A proper understanding of this interaction has to take into account its stochastic nature. We recorded voltage and calcium from spiking–bursting neurons and performed a statistical study of the correlation between both variables. We found a rich repertoire of time scales and correlated and uncorrelated parameters. We expect our results to constrain future modelling of spike bursting.

Keywords: Voltage–calcium correlation; Leech heart CPG; Calcium dye fluorescence

1. Introduction

Widely acknowledged as the director ion, the calcium ion Ca^{2+} controls excitability, neurotransmitter release and plasticity [4]. Calcium entering from voltage-dependent channels or released from internal stores controls conductances responsible for slow after hypolarization or depolarizing after potentials following spikes. These potentials, in turn, modify the neuronal firing. In effect, the slow dynamics of calcium and its interaction with the neuron's electrical responses is a form of short-term memory. This memory can be used, for example, as a delay in the computation of motion detection [2], to adapt to stimulus statistics [7,1] or to intervene in synaptic integration by affecting the shape of backpropagating action potentials [6]. Calcium is also a player in the global dynamics of the neuron. It contributes to generate complex patterns of spiking–bursting activity and can also affect the reliability of particular features of the electrical response [3]. These two effects, short-term memory and a fundamental role in the single-neuron dynamics, make calcium a major player in the network dynamics. We, therefore, expect calcium to play a role in the regulation of rhythms in the inhibitory loops found in many central pattern generators, in adaptation to statistics or more generally in the processing of recurrent circuits as predicted by realistic conductance-based models [8,13]. The many computational capabilities arising from the interaction between slow and fast dynamics are best studied in the identified neurons of invertebrates.

The leech is an ideal system to study the roles of calcium in network activity. Calcium imaging experiments in the leech have been used to study the role of calcium dynamics in dendritic processing in the AP neuron [14]. The heart interneurons HN, responsible for the CPG of heart beat control [12], have been the focus of intensive calcium imaging studies. The modulation of spike-mediated synaptic transmission by presynaptic background Ca^{2+} [10] and the relationship between the spatio-temporal patterns of Ca^{2+} changes and the slow wave changes in the voltage dynamics [9] are the two main results of these studies. In spite of several modelling efforts, the role of calcium dynamics in shaping membrane potential dynamics is still largely unknown. To further our understanding of the interplay between calcium and voltage dynamics, we have performed a statistical analysis of 50–100 s long recordings of simultaneous calcium and voltage activity in sensory neurons, interneurons and motoneurons. The analysis reveals several time scales and determines which voltage and calcium parameters best describe the correlation.

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2. Materials and methods

Medicinal Leeches (Hirudo medicinalis) were obtained from a German supplier (Zaug GmbH) and maintained in artificial pond water at 15° C in natural light. Standard techniques were used [11]. In brief, ganglia one to seven were dissected from the anesthetized animal and pinned ventral side up in standard extracellular solution. Relevant neurons were identified from their position in the ganglia and firing characteristics. The neurons were visualized using 40× water immersion objectives, under a Zeiss Axioskop FS 2 microscope. Intracellular recordings were made using a single quartz microelectrode filled with 4 M potassium acetate and potassium chloride, and pulled to resistances of 40–80 M. Signals were amplified using an Axoclamp 1 A amplifier. Calcium Green-1 was delivered into the neuron using a hyperpolarizing current of 0.5 nA for about 5 min. Calcium images were acquired using a NeuroCCD system. Statistical analysis was performed using custom-built software in Matlab programming language.

3. Results

Fig. 1B shows the somatic voltage in interneuron HN and the simultaneous fluorescence changes obtained with the calcium dye Calcium Green-1 from the region of interest indicated with a closed loop in Fig. 1A. Eye inspection already indicates that the relationship between calcium and voltage is complex compared to those found in sensory neurons (sensory cells known as N cells, for example, show a clean one-to-one correspondence between single spikes and calcium changes; data not shown).

To better understand the relationship between calcium and voltage dynamics, we calculated the correlation function between the two variables. Fig. 2 gives the probability density for calcium–voltage correlation peak values and delays for the motoneuron HE (solid line) and for interneuron HN (dashed line). To distinguish between interburst and intraburst characteristics, we have calculated correlation peak values and delays for whole recordings (top row) and single bursts (bottom row). Single burst correlations are computed as the normalized cross correlation between the calcium signal and the voltage trace in a time window starting 1 s before the first spike and ending 1 s after the last spike for each burst. Whole recording correlations take into account longer time windows comprising 2–10 bursts. Both signals are low-pass filtered before computing correlations. While whole recording correlations contain slow frequencies corresponding to the biphasic rhythm driving the leech hearts, single burst correlations only comprise fast components corresponding to spiking activity.

Ca^{2+} signals were spatially averaged in the proximal portion of the primary neurite, and temporally low-pass filtered before being analyzed. The remaining burst-to-burst calcium variability is thought to reflect the variability in the burst structure, i.e. in the profile of the instantaneous firing frequency along the burst. This idea is supported by the single burst correlation analysis, where much higher correlation peaks where observed between corresponding Ca^{2+} and V low-pass filtered signals than when they were randomly shuffled. Furthermore, Ca^{2+} rising slopes lagging the corresponding local increases in firing frequency can be detected by the naked eye in recordings shown in Fig. 1B. When a softer low pass filter was applied to the Ca^{2+} signal, almost one-to-one correspondence of Ca^{2+} peaks to individual spikes could be observed.

In general, HE cells show a stronger correlation between the voltage and the calcium dynamics, especially when

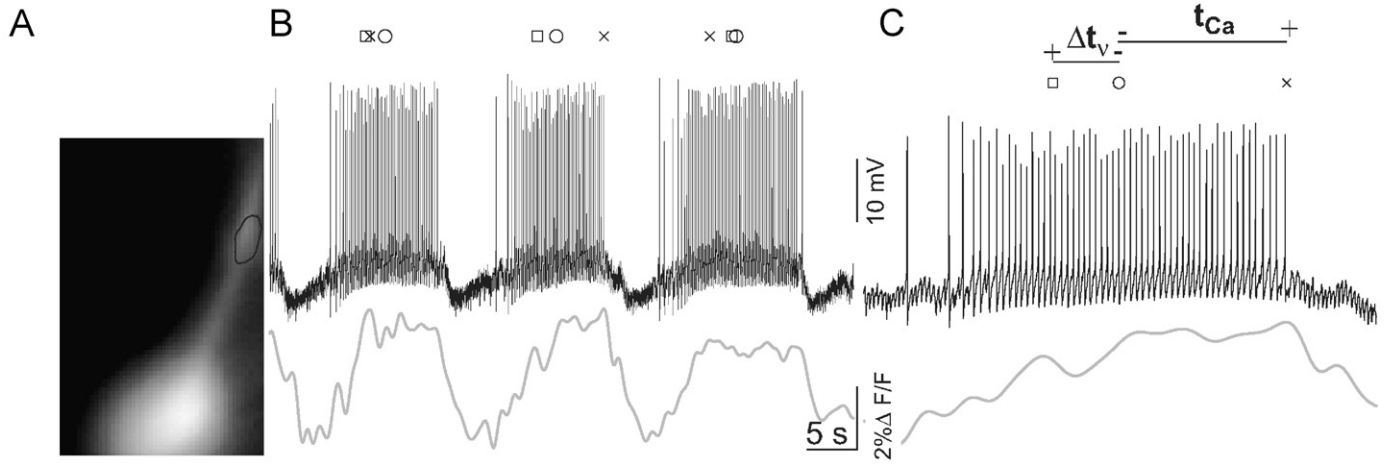

Fig. 1. Simultaneous recording of calcium dye fluorescence and membrane potential for the HN neuron. (A) Single frame showing the calcium dye fluorescence. Black closed loop indicates the region of interest in which we averaged the intensity pixel values for the calculation of a signal proportional to local calcium. (B) Membrane potential (top trace) and calcium (bottom trace). Symbols on top of the bursts indicate: ○ median spike, □ frequency maxima, × calcium maxima . (C) Enlarged view of the middle burst in (B). Lines on top of the voltage trace denote time intervals used in the analysis. Δt_{Ca} is the time difference between the calcium maximum and the median spike for each burst. Δt_v is the time difference between the maximum frequency and the median spike.

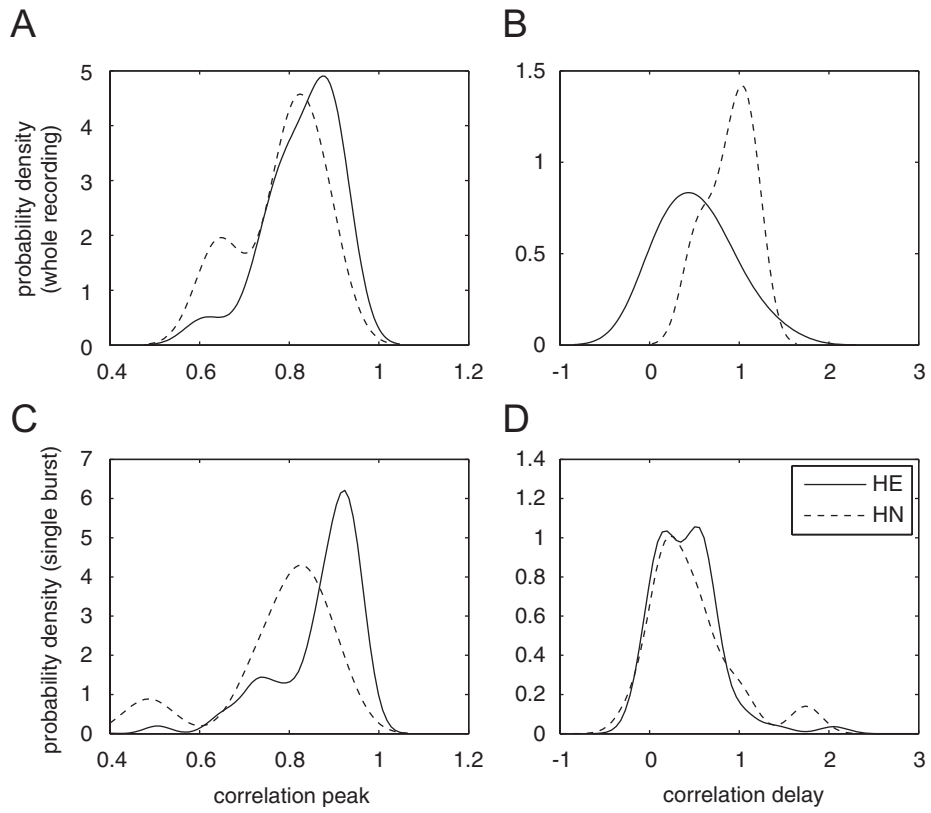

Fig. 2. Probability density for calcium–voltage correlation peaks (A,C) and corresponding delays (B,D) for HE (solid line) and HN (dashed line). Top row corresponds to whole recording correlations, while the bottom row to single burst correlations.

single burst correlations are considered, Fig. 2A and C. This stronger correlation coexists with a more variable correlation delay (wider curve for HE in Fig. 2B and D). The correlation between calcium and voltage depends upon the length of the traces: for instance, if single bursts are taken into account, the correlation peak occurs at a shorter delay (compare panels B and D of Fig. 2) than what observed when longer traces are used. We interpret this phenomenon as evidence for the interplay between calcium and voltage on multiple time scales: in the whole recording correlation analysis the interactions between voltage and calcium due to different cell mechanisms add together and the net result is a delay of calcium centered around 0.5 s for HE and 1 s for HN. On the other hand when we restrict the analysis on the bursting phase the coupling between V and Ca^{2+} is implemented with a different combination of cell mechanisms, resulting in a faster interaction.

The correlation functions show the more general characteristics of the time-scales in the relation between calcium and voltage. To find which are the key parameters that best describe the correlation between calcium and voltage, we performed a large set of statistical comparisons. Fig. 3 shows some of the most representative findings. The time corresponding to a maximum of calcium in a burst, Δt_{Ca}, measured from the time corresponding to the median spike (see Fig. 1C), is not correlated to the time of maximum spike frequency, Δt_v, also measured from the median spike, Fig. 3A. This is particularly interesting in view of the fact that the maximum value of the calcium correlates with the maximum spike frequency, Fig. 3B. Burst duration might be thought to have an impact on the maximum value of calcium but it does not for these spiking–bursting neurons, Fig. 3C. For these bursting neurons the dynamics of voltage and calcium are surely correlated, but with wide degrees of freedom. Our analysis reveals that the interdependence between the two variables concentrates in some subspace, while in other subspaces they are free to behave almost independently.

4. Discussion

Many deterministic models relating calcium and voltage can be compatible with averaged experimental data. To distinguish among them, one needs to compare their stochastic versions to statistical data like the one presented in this paper for spike bursting. Stochasticity, when the corresponding deterministic equations are nonlinear, can affect different parameters in very different ways. This translates into correlated and uncorrelated features in our analysis. Our statistical analysis of experimental data is an important first step towards a more complete model of spike bursting.

Further analysis is needed to clarify more aspects of the relationship between calcium and voltage. For example, the participation of calcium in the neuronal dynamics can regulate the timing precision of action potentials in specific

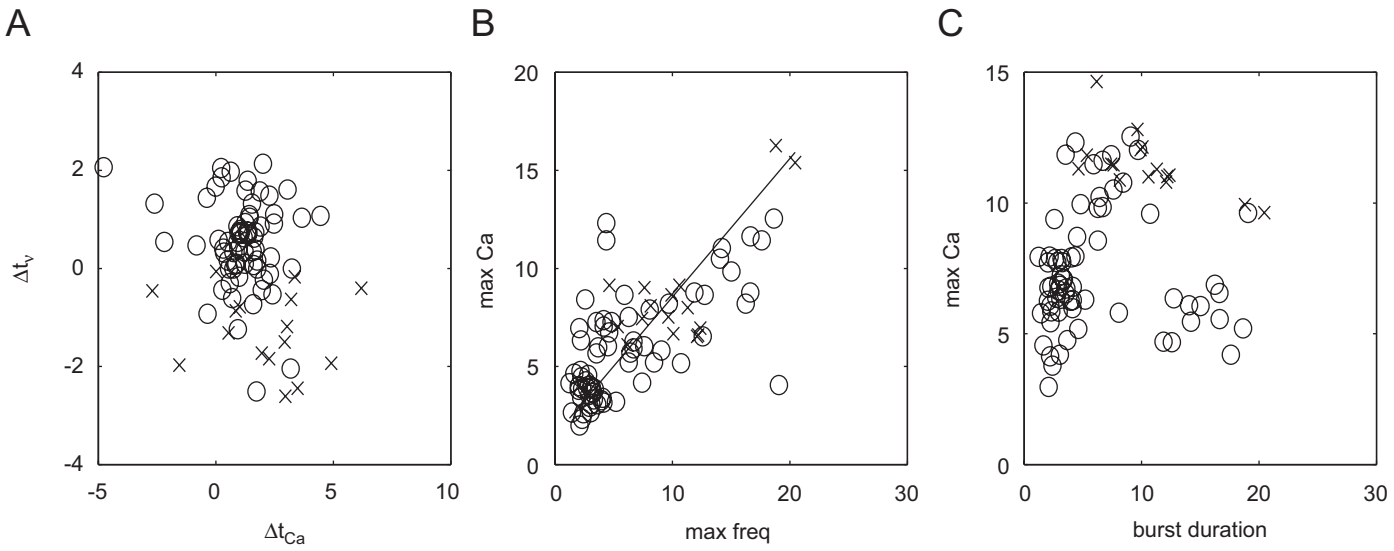

Fig. 3. Correlated and uncorrelated parameters in the relationship between calcium and voltage for HE and HN neurons. (A) Time for maximum calcium, Δt_{Ca}, is not correlated with time of maximum spike frequency Δt_v, $p>0.01$. (B) Maximum calcium value is correlated to maximum spike frequency, $p<10^{-13}$. (C) Maximum calcium value is not correlated with burst duration, $p>0.1$. Different symbols indicate different cell types: ○ HE, × HN. p values and correlation values are computed regardless of the cell type since no significant difference was found between them.

regions of the burst [3]. Recent experimental work by our group shows the presence of regions of focalized timing precision in bursting activity [5]. Future work will test the relevance of calcium for this timing precision. Also, here we did not consider spatial patterns of activity. We expect diffusion to influence the time scales observed. Extension of the present analysis in the time domain to the full spatiotemporal domain will clarify this issue.

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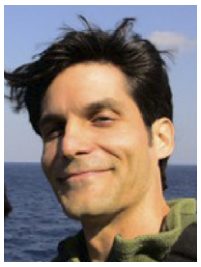

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