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Applications Note

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CaSiAn: a Calcium Signaling Analyzer tool

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Abstract

Summary: Ca^{2+} is a central second messenger in eukaryotic cells that regulates many cellular processes. Recently, we have indicated that typical Ca^{2+} signals are not purely oscillatory as widely assumed, but exhibit stochastic spiking with cell type and pathway specific characteristics. Here, we present the **Ca**lcium **Si**gnaling **An**alyzer (CaSiAn), an open source software tool that allows for quantifying these signal characteristics including individual spike properties and time course statistics in a semi-automated manner. CaSiAn provides an intuitive graphical user interface allowing experimentalists to easily process a large amount of Ca^{2+} signals, interactively tune peak detection, revise statistical measures and access the quantified signal properties as excel or text files.
Availability and implementation: CaSiAn is implemented in Java and available on Github (https://github.com/mmahsa/CaSiAn) as well as on the project page (http://r3lab.uni.lu/web/casa).
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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Ca^{2+} is a universal intracellular messenger that translates extracellular signals into intracellular responses often through sequences of concentration spikes. The versatility of Ca^{2+} is afforded by a broad spectrum of signals allowing for well-controlled activation of distinct pathways including proliferation, gene expression, metabolism, muscle contraction, learning and memory as well as apoptosis (Berridge *et al.*, 2000; Clapham, 2007). The often rather regular appearing spiking behaviour has led to the perception that Ca^{2+} signaling transmits information in a frequency encoded manner as exemplified experimentally for gene expression efficiency of NFAT, OCT4 and NF-κB (Dolmetsch *et al.*, 1998).

More recently, we have shown that Ca^{2+} signals of diverse cell types exhibit a non-negligible stochastic character consisting of random sequence of concentration spikes rather than deterministic oscillations (Dupont *et al.*, 2008; Skupin *et al.*, 2008). The randomness of spiking originates from the molecular fluctuations of individual Ca^{2+} release channels which are translated onto the level of the cell by Ca^{2+}-induced Ca^{2+} release (CICR; Skupin and Falcke, 2009). The spatial coupling and self amplification of random events by CICR introduce an hierarchical signaling systems with a linear relation between the variability measured by the standard deviation (SD) σ and the average period T_{av} of interspike intervals (ISIs; Skupin *et al.*, 2008, 2010). Interestingly, this relation is cell type and pathway specific indicating that cells do not only encode information in the frequency, but also may use higher statistical moments for information processing (Skupin and Falcke, 2009; Thurley *et al.*, 2014).

Understanding this signaling mechanism relies on a statistically firm characterization of signal properties. Available tools for such an automatic analysis of spiking data are typically tailored towards spiking dynamics of neuronal membrane potentials (Cajigas *et al.*, 2012) but do not reflect the specific needs for Ca^{2+} signals that often exhibit more noise and slow underlying trends. A recent approach

addresses Ca^{2+} spike recognition by Matlab scripts (Russo *et al.*, 2013), but without an interactive graphical user-interface (GUI), limiting the application to computational scientists. In particular, these approaches do not allow for an intuitive optimization of signal analysis nor a systematic analysis of the σ-T_{av} relation.

To address this need, we here introduce the **Ca**lcium **Si**gnaling **An**alyzer (CaSiAn)–an intuitive and interactive Ca^{2+} signaling analyser tool enabling an efficient analysis of large time course datasets in a semi-automated way.

2 Materials and methods

CaSiAn is implemented in Java to enable platform independence and has an intuitive GUI allowing to control easily all analysis related parameters and files. Once Ca^{2+} time course data extracted from fluorescent images is loaded, the CaSiAn workflow enables (i) normalization and background removal, (ii) peak and nadir detection needed for the definition of ISIs, (iii) determining a wide range of signal properties including spike amplitudes and widths, (iv) interactive analysis of the σ-T_{av} relation and finally (v) export of signal characteristics as CSV or Excel files as summarized in Supplementary Figure S1 and detailed in Supplementary Material and Figures S2–S9. The interactive CaSiAn GUI (Supplementary Fig. S10) allows for fast data screening, adding or removing of peaks and nadirs and exclusion of specific signals. From the curated data, CaSiAn determines a wide range of signal properties including spike amplitude (AMP), spike width (SW), area under the spike baseline (AUS_{sp}), further shape properties (TPP, IR, DR detailed in Supplementary Material and Supplementary Figs S4–S8) and individual ISIs (Supplementary Fig. S1D). The extracted individual ISIs allow for quantifying the SD σ and the mean period T_{av} for each spike pattern. To investigate the cell type and pathway specific linear relation between these two quantities, CaSiAn offers to plot this relation with the corresponding linear fitting in an interactive popup window allowing for an in-depth analysis even for non-computational scientists.

3 Results and discussion

As an application demonstration of the CaSiAn tool, we imaged Ca^{2+} signals of individual C8–D1A mouse astrocytes stimulated by extracellular adenosine triphosphate (ATP) inducing IP_3 induced Ca^{2+} spiking (Supplementary Material, Section S6.1). To investigate the effect of extracellular stimulation strength, we treated cells first with 10 µM ATP for 27 min and subsequently increased the concentration to 80 µM ATP as indicated in Supplementary Figure S1B by red and blue arrows, respectively. After signal baseline removal, the signals of 250 individual cells were split into two separated analysis periods and individual ISIs were detected automatically for the two measuring periods (Supplementary Fig. S1C). After this detection of peaks and nadirs, CaSiAn calculated all signal properties illustrated in Supplementary Figure S1D as well as σ and T_{av} for each spike train. To evaluate the different signal properties, the dependence between σ and T_{av} are plotted by the CaSiAn routine and subsequently filtered for six ISIs needed for stable quantification of σ. The resulting linear relations can be further analyzed by correlating individual (σ, T_{av}) values with the corresponding temporal signals. The statistical analysis of the two different stimulation concentrations shows that the higher stimulation with 80 µM ATP leads to significantly increased average periods (T_{av}), average amplitudes and average spike widths (Supplementary Fig. S11). In addition, comparing AUS_{sp} values reveals an increased average Ca^{2+} concentration in cells for the 80 µM ATP treatment mainly caused by the increase in SW. The increase in T_{av} and the smaller slope of the σ-T_{av} relation (Supplementary Fig. S11) for the higher stimulation period indicates that cells do not transmit information by pure oscillatory properties but potentially also by other mechanisms like frequency-amplitude encoding (De Pittà *et al.*, 2009) or modified variability of the signal (Thurley *et al.*, 2014).

As another application, we investigated Ca^{2+} dynamics within zebrafish brains during epileptic seizure genesis. For this purpose, Tg(actb2:GCaMP6f) 5 days post-fertilization zebrafish larvae expressing the Ca^{2+} sensitive GCaMP6 fluorophore were incubated either with 1 mM picrotoxin (PTX) or vehicle control (1% DMSO) and imaged on a laser scanning confocal microscope for 45 min with an imaging rate of 5 Hz (Supplementary Material, Section S6.2). After loading the extracted time course into CaSiAn, we obtained the properties of signals from three brain regions and performed a correlation analysis. The PTX treatment led to a slow increase of spike amplitudes over time in all three considered brain regions, followed by a sharp jump in Ca^{2+} signal which may relate to seizure activity (Supplementary Fig. S12). Together with the decreasing spike width before the global Ca^{2+} spikes, these increased amplitudes reflect the increasing synchronization of neuronal activity. We next analyzed the correlation between the released Ca^{2+} estimated by AUS_{sp} and the corresponding ISI and found significant correlations for all brain regions (Supplementary Table S1) reflecting the PTX induced seizure like dynamics. This driven synchronization is further indicated by the strong correlation between successive ISIs in contrast to spontaneous spiking behavior (Supplementary Fig. S12; Skupin and Falcke, 2009).

Overall, CaSiAn is a powerful tool to investigate heterogeneous Ca^{2+} time course data in diverse model systems in a high-throughput manner. Its interactivity and easy to apply statistical analysis framework will allow for new insights in many different signaling pathways.

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Conflict of Interest: none declared.

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