and by modeling. The role of luminal calcium has not been investigated with that much detail although it has been found that it is relevant for signal termination in the case of Ca^{2+} release through Ryanodine Receptors. In this work we present the results of observing the dynamics of luminal and cytosolic Ca^{2+} simultaneously in Xenopus Laevis oocytes using two dyes that have their peaks of emission at different wavelengths. Through a continuous photorelease of caged IP3 we are able to evoke a series of global and localized signals in this system. The analysis of such signals allows a characterization of the extent to which distant regions can be coupled via the dynamics of luminal calcium. In this way we expect to advance into answering whether luminal calcium can generate the global feedback mechanism that is necessary to explain the robustness of IP3R-mediated calcium spikes as a signaling tool in spite of the inter-spike interval randomness.

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$InsP_3R$ Activation Facilitates Ca^{2+} Wave Propagation in Ventricular Myocytes

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The contribution and significance of sarcoplasmic reticulum (SR) Ca^{2+} release driven by $InsP_3$-R activation (IP3ICR) in cardiac excitation-contraction coupling (ECC) is still a matter of debate. In particular it's role in the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism in non-hypertrophic ventricular myocytes seems to be ambiguous. Here we characterize the contribution of IP3ICR in Ca^{2+} wave propagation driven by CICR in ventricular myocytes. We hypothesized a functional crosstalk and/or cooperativity between both SR-Ca^{2+} release channels, e.g. IP3ICR may trigger or facilitate CICR via RyR activation and/or RyR sensitization. IP3ICR was activated in a highly targeted way by UV-flash photolysis of a membrane-permeant caged $InsP_3$ while simultaneously imaging Ca^{2+} with laser-scanning confocal microscopy in ventricular myocytes acutely isolated from mouse hearts. Recently, it has been shown that cellular ECC remodeling under pathophysiological stress leads to increased expression of $InsP_3$-Rs and IP3ICR. To boost this situation to the extreme and in order to unmask a potential role of IP3ICR in ECC we used an $InsP_3$-R overexpressing mouse model. Photolytic $InsP_3$ release resulted in an immediate increase in Ca^{2+} wave propagation (from 63 ± 7.3 µm/s in control to 79 ± 3.7 µm/s after flash, n=10). This response was sensitive to the $InsP_3$-R blocker Xestospongin C and was not seen in WT mice. After repetitive photolytic $InsP_3$ release in a time window of >5 min, Ca^{2+} wave propagation reverted back to control. In addition, spontaneous Ca^{2+} wave appearance decreased or dissapeared. This suggests that Ca^{2+} wave propagation under conditions of high functional $InsP_3$-R expression are accelerated by regional RyR sensitization mediated by IP3ICR. However, over long term RyR Ca^{2+} desensitisation and SR-Ca^{2+} store depletion by $InsP_3$-Rs openings may lead to Ca^{2+} wave termination. Supported by SNF and Novartis Res. Foundation.

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Characterization of Calcium Release Events Evoked by $InsP_3R$ Activation in Intact and Permeabilized Atrial Myocytes

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In cardiac myocytes Ca^{2+} release events based on the intracellular second messenger inositol-1,4,5-trisphosphate receptor ($InsP_3R$) activation are not conclusively characterized. This Ca^{2+} release mechanism may play a significant role under pathophysiological conditions, e.g. atrial arrhythmogenicity. In this study we characterize $InsP_3$-induced SR-Ca^{2+} release events (Ca^{2+} puffs) and spontaneous SR-Ca^{2+} release events (Ca^{2+} sparks) based on RyRs openings in atrial myocytes. Local Ca^{2+} release events were examined in intact or permeabilized atrial myocytes acutely isolated from transgenic mouse hearts overexpressing $InsP_3$Rs by using rapid 2-dimensional confocal imaging (150 fps). $InsP_3$Rs were activated by rapid superfusion with $InsP_3$ (permeabilized cells) or by ET-1 (intact cells). Xestospongin C was used as antagonist. Ca^{2+} puffs were solely appearing in the presence of intracellular $InsP_3$. However, Ca^{2+} puffs may trigger Ca^{2+} sparks and both likely coexist, making the event separation and analysis from confocal images challenging. We compare two analytical approaches for quantitative Ca^{2+} event analysis based on different mathematical formalisms. One procedure operates with a spark as the rejection of the null hypothesis that the fluorescence change is noise. The second performs pixel-by-pixel fitting of the fluorescence signal and reconstructs underlying Ca^{2+} events via a clustering algorithm. Although rapid confocal imaging of the entire cell cross section always suffers from low signal-to noise ratio, this approach gives a more detailed and representative view of local Ca^{2+} events. Our new formalism for Ca^{2+} event analysis allows us to separate and characterize micro Ca^{2+} events (Ca^{2+} puffs, Ca^{2+} sparks) by their absolute number, amplitude, signal mass, area, $FWHM_X$, $FWHM_Y$ and kinetics using high speed imaging. We found that Ca^{2+} puffs exist as specific events in atrial myocytes, which could not be seen previously. Supported by SNF, SciEx and Novartis Res. Foundation.

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Extracting Detailed Ca^{2+} Signaling Information from Noisy Confocal Images

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Analysis of Ca^{2+} signaling in cardiac cells is often a trade-off between acquisition speed and signal-to-noise ratio. This becomes especially apparent in fast 2D scanning or when recording fluorescence signals from the sarcoplasmic reticulum, for example. Methods have been developed to remedy this via 'denoising' the image by fitting each pixel with a transient function. So far, adoption of such methods has been hindered by a number of limitations (e.g., inability to fit local, concurrent and consecutive events) and the limited availability of a customizable implementation.

Here we present a novel method for performing per-pixel denoising of confocal frame- and linescans. Our algorithm permits the extraction of spatiotemporally overlapping events (e.g., a spark occurring during the decaying phase of a Ca^{2+} wave) and is able to detect various different types of events within a pixel time course. The method estimates a non-constant baseline for each pixel, negating the necessity of using background regions or self-ratio methods prior to performing the analysis. Furthermore, by applying a clustering algorithm, detected single-pixel events are grouped into physiologically relevant events spanning multiple pixels (sparks, waves, puffs,transients, etc.), from which traditional parameters such as FDHM, FWHM, amplitude, wave speed, rise and decay times, can be easily extracted.

The method has been implemented as a cross-platform open source software with a comprehensive and easy to use graphical user interface. We have applied our method to analyzing linescans of repetitive sparks from individual RyR clusters; high-speed (150 frames/sec) framescans containing alterations in Ca^{2+} release events in atrial myocytes; and parallel analysis of Ca^{2+} release dynamics in the sarcoplasmic reticulum and cytosol.

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Increased Accuracy of Calcium Spark Parameter Detection using High-Speed Confocal Microscopy

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High-speed confocal microscopy is a method now commonly used to investigate subcellular calcium release events, but whose potential is rarely used fully without an effective standard analysis method.

Here a detailed evaluation of a standalone analysis package for line-scan and X-Y images is presented. The algorithms used are based on a one-dimensional stationary wavelet transform. Computer generated images mimicking the properties of real recordings were used to assess the accuracy of the algorithms.

On line-scan images the minimum usable detection amplitude for stereotypical calcium sparks at a sensitivity of ca. 80% was found to be 0.085. Sparks of amplitude ($\Delta F/F_0$) 0.15 and above are detected with a sensitivity of at least 98%. Due to the large number of points in a calcium spark, specificity exceeds 95% in all cases, enabling automatic analysis without manual correction. Furthermore, the temporal characteristics of sparks can be determined with a very high accuracy.

On series of X-Y images the cell orientation and its sarcomeric structure is determined. Here, the minimal detection amplitude for generated sparks with 2 µm FWHM is 0.08 at a sensitivity level of 94% with a specificity level exceeding 98%. Using optimal detection parameters, sparks of 0.2 amplitude are found with a sensitivity of 100%. A separation distance of 3.6 µm is required between the centers of two sparks. The high temporal resolution makes it possible to study the spatiotemporal evolution of a calcium release event in 2D since events found on subsequent images at identical locations are automatically linked to each other.

As the levels of detection parameters show, the assessed toolset, available upon request, is highly effective in the analysis of muscle calcium release events and may be useful for the analysis of other fluorescent calcium images.