Illuminating the fundamental structure of elementary Ca^{2+} signals with a combination of realtime nanoscopy and CaCLEAN in living cardiac myocytes

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In cardiac myocytes, the cellular Ca^{2+} transient determines the performance of the entire heart and is established by the recruitment of elementary Ca^{2+} release signals, the so-called Ca^{2+} sparks[1]. For more than 25 years sparks are believed to originate from clusters of the principal Ca^{2+} release channel, the ryanodine receptor (RyR) on the sarcoplasmic reticulum, [2]. Surprisingly, super-resolution imaging of immunolabelled fixed cardiac myocytes indicated an average density of RyR clusters of ~2.2/μm^3 [3] suggesting cluster distances well below the spread of an individual Ca^{2+} spark (FWHM ~2.8 μm). A novel instant SIM (iSIM, VisiTech Ltd, Sunderland, UK) microscope allows super resolution imaging (<130 nm lateral and <270 nm axial) at unprecedented acquisition rates (~175 frames/s, @1840x1024 pixels with a Hamamatsu ORCA Flash4 sCMOS camera). This realtime nanoscopy together with our computational methodology recently introduced [4] combining astronomical imaging processing with our knowledge of release and diffusion of Ca^{2+} enabled us – for the first time – to identify fundamental nanoscopic Ca^{2+} release bursts CRB) that build up the microscopic Ca^{2+} spark. On average, around 6 of such CRBs fire during a normal Ca^{2+} spark. Physiological up or down regulation of the spark's amplitude by e.g. dissociation of the RyR accessory protein FKB506 or phosphorylation of the RyR results in reduced (~4.7) and increased (~9) numbers of contributing CRBs, respectively. STED microscopy of fixed and immunolabelled ventricular myocytes clearly supported our functional findings in that microscopic (i.e. confocal) RyR clusters clearly comprised many (up to >10-15) RyR sub clusters. Contributing CRBs fire in succession and spatial proximity appears to determine the particular activation order. Registration of a longer time series of spontaneous Ca^{2+} sparks and their associated CRBs reveals a well-ordered distribution of release sites reflecting the RyR distribution. In this study we combined innovative realtime nanoscopy with the novel algorithmic approach CaCLEAN to unravel the fine-structure of elementary Ca^{2+} release signals, Ca^{2+} sparks. We established – in contrast to the current view – that sparks indeed comprise a multitude of CRBs that build up the microscopic Ca^{2+} transient. We believe that individual RyR clusters represent the morphological correlate to these CRBs. Physiological and pathological modulation of RyR results in a graded recruitment of CRBs to the Ca^{2+} spark and results in its altered properties.

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