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Elementary calcium release events from the sarcoplasmic reticulum in the heart

Didier X.P. Brochet[*,§], **Dongmei Yang**[†], **Heping Cheng**[‡], and **W. Jonathan Lederer**[*]

[*]Center for Biomedical Engineering and Technology (BioMET), Department of Physiology, University of Maryland School of Medicine, University of Maryland, Baltimore, MD 21201

[†]Laboratory of Cardiovascular Sciences, National Institute on Aging, Baltimore, MD 21224

[‡]Institute of Molecular Medicine, National Laboratory of Biomembrane and Membrane Biotechnology, Peking University, Beijing 100871, China

Abstract

Ca^{2+} release events underlie global Ca^{2+} signaling yet they are regulated by local, subcellular signaling features. Here we review the latest developments of different elementary Ca^{2+} release features that include Ca^{2+} sparks, Ca^{2+} blinks (the corresponding depletion of Ca^{2+} in the sarcoplasmic reticulum (SR) during a spark) and the recently identified small Ca^{2+} release events called quarky SR Ca^{2+} release (QCR). QCR events arise from the opening of only a few type 2 ryanodine receptors (RyR2s) - possibly only one. Recent reports suggest that QCR events can be commingled with Ca^{2+} sparks and may thus explain some variations observed in Ca^{2+} sparks. The Ca^{2+} spark termination mechanism and the number of RyR2 channels activated during a Ca^{2+} spark will be discussed with respect to both Ca^{2+} sparks and QCR events.

Keywords

Ca^{2+} blink; Ca^{2+} spark; heart; quarky SR Ca^{2+} release; ryanodine receptor

Introduction

Cardiac contraction underlies the pumping of blood needed to perfuse tissue and thereby deliver nutrients and oxygen. The cardiac action potential (AP) regulates contraction through the process of excitation-contraction (EC) coupling. Invaginations of the plasma membrane extend the surface membrane into the cardiomyocytes through the T-tubule (TT) network found in mammalian ventricular myocytes enabling the AP to control EC coupling throughout the cell. The intracellular Ca^{2+}-rich organelle, the sarcoplasmic reticulum (SR) is organized so that the primary Ca^{2+} release component, the junctional SR (jSR) forms a thin pancake like structure that wraps around the TT and that remains within 15 nm of the TT membranes. The Ca^{2+} release channels (RyR2s) reside in the jSR and span the gap (the subspace) between the TT and jSR membranes. During EC coupling, activation of L-type Ca^{2+} channel (LCC) by the AP enables a small "puff" of Ca^{2+} to enter the subspace and activate RyR2s. The activated RyR2s open and permit Ca^{2+} to move from the jSR lumen into the cytosol. The local $[Ca^{2+}]_{subspace}$ bathes the other RyR2s in the same subspace and they in turn are activated. This process is called Ca^{2+}-induced Ca^{2+}-release (CICR) and it

[§]To whom correspondence should be addressed: Didier X.P. Brochet, Center for Biomedical Engineering and Technology (BioMET), Department of Physiology, University of Maryland School of Medicine, University of Maryland, 725 W. Lombard Street, Baltimore, MD 21201 USA, Tel: 410-706-1125, Fax: 410-706-8184, dbrochet@umaryland.edu.

amplifies the initial Ca^{2+} entry into the cytosol. The measured increase of cytosolic $[Ca^{2+}]_i$ due to the activation of RyR2s in one jSR is seen as a Ca^{2+} spark. The unit of such Ca^{2+} release, including the LCC, the jSR with its cluster of RyR2s and local structures are called a Ca^{2+} release unit (CRU). Depending on species, 70 to 90% of the Ca^{2+} released during EC coupling is through the SR (Bassani *et al.*, 1994). CICR is therefore an important part of the EC coupling. During EC coupling, a $[Ca^{2+}]_i$ transient is actually composed of thousands of Ca^{2+} sparks (Cannell *et al.*, 1994; Lopez-Lopez *et al.*, 1995; Cannell *et al.*, 1995).

Ca^{2+} spark

Ca^{2+} sparks can arise when the LCC are opened by depolarization, when local $[Ca^{2+}]_i$ is elevated by some other mechanisms but they can also open "spontaneously". Such spontaneous openings occur because each RyR2 has a finite probably of opening even when the diastolic $[Ca^{2+}]_i$ is low. While that probably is low, it is sufficient in rat heart cells to underlie a spontaneous Ca^{2+} spark rate of about 100 per cell per second. In all cases of Ca^{2+} spark occurrence, there is a component of CICR. Given that $[Ca^{2+}]_i$ in the subspace is elevated by the opening of a local LCC or the probabilistic RyR2, this elevated subspace $[Ca^{2+}]_{subspace}$ increases the likelihood that another RyR2 opens. In this manner CICR is a key element in the generation of a Ca^{2+} spark (Prosser *et al.*, 2010).

Ca^{2+} sparks typically are small with a diameter at the peak $[Ca^{2+}]_i$ of the spark characterized by the "full width at half-maximum" or FWHM (2 –2.5 μm). They also have a variable amplitude ($\Delta F/F_0$ up to about 4) and a short duration (half time of decay of about 20–30 ms, (Cheng *et al.*, 1993)), suggesting a strong termination mechanism (Sobie *et al.*, 2002; Williams *et al.*, 2011)(Fig 1A). The kinetics of Ca^{2+} sparks reflect the duration of release, the local diffusion of the released Ca^{2+} and the cytosolic buffering of Ca^{2+}. In the steady-state, the Ca^{2+} that is released by Ca^{2+} sparks is balanced by restorative mechanisms such as reuptake of Ca^{2+} into the SR by the SR/endoplasmic reticulum (ER) Ca^{2+}-ATPase2a (SERCA2a) and extrusion of Ca^{2+} from the cell into the extracellular compartment by the plasmalemmal sodium-calcium exchanger and the plasmalemmal Ca^{2+}-ATPase. The mitochondria take up virtually no Ca^{2+} (Andrienko *et al.*, 2009).

Ca^{2+} blink

In cardiac cells, even if the volume of the SR accounts only for a few percent of the total volume of the cell, it has a major role, essentially as a dynamic Ca^{2+} store. It releases Ca^{2+} when the RyR2s open and reacquires Ca^{2+} by means of SERCA2a. The $[Ca^{2+}]_{SR}$ is around 1 mM, about 10,000 times higher than $[Ca^{2+}]_i$ in the cytosol. The SR is composed of two compartments: the jSR at the z-bands and the free SR (fSR) that connects the jSRs. In rabbit heart cells, the fSR is connected to the jSR by 4–5 diffusional strictures of only 30 nm in diameter (Brochet *et al.*, 2005), slowing down Ca^{2+} diffusion into the jSR during a spark and thereby allowing isolation of Ca^{2+} signaling in the jSR (also relevant for spark termination). The fSR and jSR are interconnected within the cell and form a single network that includes the ER and the nuclear envelope (Wu and Bers, 2006). The fSR forms a diffuse network composed of interconnected narrow tubules whereas the jSR is composed of enlarged cisternae (as much as about 592 nm in diameter and 30 nm in thickness). The RyR2s in the jSR face the TT and LCC. The number of RyR2s at a jSR is comprised between 30 and 300 and forms a 2D paracrystalline array(Franzini-Armstrong *et al.*, 1999) although recent findings (Baddeley *et al.*, 2009; Hayashi *et al.*, 2009) suggest that these arrays are incomplete and may arise from an assemblage of incomplete subclusters. The jSR not only contains the RyR2s but also the high capacity Ca^{2+} buffer calsequestrin (Franzini-Armstrong, 1980).

The use of the low affinity Ca^{2+} indicator fluo-5 N which can be targeted to the SR by "loading protocols" has allowed the visualization of Ca^{2+} within the SR (when the dye can be accumulated preferentially in the jSR) (Kabbara and Allen, 2001; Shannon *et al.*, 2003). The visualization of the depletion of Ca^{2+} within a jSR during a spark, called Ca^{2+} blink, was possible because of the diffusional strictures between the fSR and jSR (Brochet *et al.*, 2005). The simultaneous visualization of sparks (on the cytosolic side) and blinks (on the intra-SR side) has allowed a better understanding of the Ca^{2+} dynamics during a spark (Brochet *et al.*, 2011). The sharpness of blinks (FWHM=1 μm), suggests that SR Ca^{2+} depletion is largely confined to a single jSR during a spark and therefore that Ca^{2+} sparks are the result of the activation of a single CRU. The amplitude of jSR Ca^{2+} depletion during a Ca^{2+} blink was about 80–85 % of the SR Ca^{2+} depletion during a full-fledged transient, indicating that the jSR was largely depleted of Ca^{2+} during a spark.

Quarky SR Ca^{2+} release (QCR) event

Activation of a single or a few RyR2s during a QCR event

The simultaneous recording of cytosolic [Ca^{2+}] and intra-SR [Ca^{2+}] was the tool needed for a more adequate understanding of the Ca^{2+} release process. This approach allowed us to examine the distinction between genuine small Ca^{2+} release events and out of focus sparks. An out of focus Ca^{2+} spark does not reveal Ca^{2+} blink signal since the jSR from which the Ca^{2+} blink signal arises is out of the plane of focus. Indeed, because of the localized FWHM of Ca^{2+} blinks, this technique has allowed the detection of very small Ca^{2+} release events (from 1/3 to 1/10 of a spark) with very rapid kinetics (t_{67}=20 ms, (Brochet *et al.*, 2011)) (Fig 1B). Interestingly, these small spontaneous events look similar to previously described sparkless Ca^{2+} release (quarks) triggered by CICR in guinea-pig under two photon photolysis of caged Ca^{2+}(Lipp and Niggli, 1998).

How can a single RyR2 be activated without activating the array of RyR2s?

The smallest of the SR Ca^{2+} release events, now identified as "quarky SR Ca^{2+} release events" or QCR events, reflect the release of Ca^{2+} from a range of RyR2s - one to a few RyR2s. This raises the question of how can a single RyR2 or a very few RyR2s be activated within a full array of RyR2s without activating the rest of the array by CICR. One possibility is that not all RyR2s behave the same way within an array of RyR2s. For example, it is possible, in principle, that there is a mixture of naive RyR2s and inactivated RyR2s that exist within a cluster of RyR2s at the jSR. If such a condition could occur, then one or a few RyR2s could be activated while the remaining RyR2s may be "pre-inactivated". However, Ca^{2+} spark properties can be fully modeled without inactivation (Sobie *et al.*, 2006). Another possibility is that RyR2s within the large array may not be activated by opening of a single (or few) RyR2 because of the weak negative allosteric effect of the binding of Mg^{2+} to RyR2 on the transition of the RyR2 to the open state (Zahradnikova *et al.*, 2010). The most likely explanation, however, is that one RyR2 may activate the RyR2 cluster with a probability significantly less than one (Williams *et al.*, 2011). Our preference for the Williams model arises because it so nicely reproduces diverse experimental findings (e.g. Ca^{2+} spark rate, variability and modulation as well as SR Ca^{2+} leak through RyR2s).

In addition to the explanation of Williams *et al.* (2011), the geometry of the RyR2s within the jSR may importantly contribute to Ca^{2+} spark features including QCR. Using EM tomography and PALM (photo-activated light microscopy), the organization of the RyR2s within the RyR2 cluster at the jSR is one that is not fully packed (Baddeley *et al.*, 2009; Hayashi *et al.*, 2009). Instead, RyR2s at a jSR appear to be grouped in large number of small arrays of RyR2s of different sizes (from 1 to >100 RyR2s) close one from another. The average RyR2s per array was 14 with larger arrays of 25 RyR2s on average and some

superclusters (association of clusters within 100 nm of each other) averaging 22 RyR2s. The physical distance between these arrays of RyR2s of different sizes may then contribute to the diversity in QCR events and Ca^{2+} sparks.

Spark initiation

The existence of these QCR events raises the question of Ca^{2+} spark initiation. As one RyR2 within a CRU opens, the probability of other RyR2s opening is increased due to CICR (Cheng and Lederer, 2008; Williams *et al.*, 2011). The amount of Ca^{2+} flux into the subspace is roughly the same as the amount of Ca^{2+} influx into the subspace due to the opening of the LCC. The probability of a Ca^{2+} spark increases significantly as additional RyR2s are activated and as the $[Ca^{2+}]_{subspace}$ increases. We imagine that QCR events would initiate Ca^{2+} sparks in a similar manner. However, it would be very difficult experimentally to observe QCR initiated Ca^{2+} sparks because of the rapidity of successive activation of RyR2s within the CRU and the signal-to-noise ratio.

During diastole and in the absence of a triggering LCC, Ca^{2+} sparks and QCR events still occur. This can happen because there is a finite opening rate of RyR2s that depends on many factors including the phosphorylation state (Valdivia *et al.*, 1995; Marx *et al.*, 2000; Guo *et al.*, 2006; Yang *et al.*, 2007), $[Mg^{2+}]_i$, RyR2 oxidation and nitrosylation states, $[Ca^{2+}]_{SR}$ and many more (Tocchetti *et al.*, 2007; Prosser *et al.*, 2011). The estimated opening rate of RyR2s under diastolic physiologic conditions is about 10^{-4} s^{-1} which corresponds to a diastolic Ca^{2+} spark rate of about 100 sparks per cell per second in rat ventricular myocytes. During diastole, Ca^{2+} sparks are initiated by the opening rate of the RyR2s. Briefly, then, Ca^{2+} sparks are initiated by LCC during an AP. As noted above, however, and as modeled by Williams *et al.* (2011), neither LCC nor RyR2s entrain Ca^{2+} sparks with 100% fidelity. QCRs arise when the Ca^{2+} sparks are not triggered.

Complex features of Ca^{2+} sparks

Mixture of activation of array and rogue RyR2s during a spark

The simultaneous measurement of spark and blink allows a more comprehensive understanding of the Ca^{2+} release process during a spark. Recent experiments have shown that the duration of Ca^{2+} release during a spark could be similar to the phase of Ca^{2+} depletion within the SR during a blink (Brochet *et al.*, 2011). Differences in the kinetics of cytosolic and SR Ca^{2+} diffusion and Ca^{2+} buffering may have led to differences in the signals. Importantly, when EGTA was increased in the buffer, the duration of the trailing part of a spark and recovering part of a blink became shorter, suggesting a CICR mechanism during the tail of a spark enabling the Ca^{2+} spark to last longer. However the rising phase of the Ca^{2+} sparks was invariant in the presence or absence of EGTA. These results suggest that during the rising phase of a spark, the main array of RyR2s produced enough Ca^{2+} to sustain the activation. Sobie *et al.* (2006) suggested that large arrays of RyR2s would be less sensitive to Ca^{2+} at low Ca^{2+} than single RyR2 but more sensitive to Ca^{2+} at high Ca^{2+}. This depended on the cooperativity of RyR2s in a cluster. The large clusters would thus flicker less at low $[Ca^{2+}]_i$ and be more constantly activated at high $[Ca^{2+}]_i$. Therefore, despite the decrease of Ca^{2+} within the jSR during a spark, rogue RyR2s could be repetitively activated during the tail of a spark, prolonging the spark duration, whereas the probability of a large array of RyR2s to be re-activated would be minimal (Fig 2). Thus QCR would have greater variability in Ca^{2+} spark duration than Ca^{2+} sparks that originate from within a cluster and that could then explain the variability observed in spark duration (90% of spark t_{67} between 25 and 95 ms (Brochet *et al.*, 2011)).

This phenomenon may also contribute to the explanation of the occurrence of rare sparks with long kinetics characterized most of the time by a peak followed by an extended plateau

(Cheng *et al.*, 1993; Xiao *et al.*, 1997; Zima *et al.*, 2008; Brochet *et al.*, 2011)(Fig 1C). The variation in the amplitude of the plateau suggests that this plateau could correspond to the maintaining of a varying number of opened RyR2s corresponding to small cluster of RyR2s (and not the main cluster anymore) for an extended period of time. Indeed, the absence of these long events in the presence of high EGTA (2 mM) further reinforces the notion of a CICR mechanism in the plateau phase of these long sparks. The similar and simultaneous variation in the amplitude of sparks and blinks during the plateau phase also indicates quick Ca^{2+} dynamics between the release and refilling of Ca^{2+}, suggesting that the SR is not fully depleted of Ca^{2+} during the plateau phase of these long events.

Spark termination and refractoriness

The possible opening of small array or rogue RyR2s on the tail of Ca^{2+} sparks suggests that I_{spark} is not a square function but rather a triangle function or decreasing function of time (Sobie *et al.*, 2002; Williams *et al.*, 2011). How can then one explain the duration of sparks and its powerful termination scheme? Several mechanisms of spark termination have been proposed over the years. These different mechanisms include the jSR Ca^{2+} depletion during a spark (Ca^{2+} blink) that could lead to reduced Ca^{2+} efflux and RyR2 activation or that may underlie deactivation of the RyR2s from the intra-SR side (Gyorke and Gyorke, 1998). Furthermore, it has been shown that addition of a Ca^{2+} buffer within the SR prolonged the spark duration, suggesting that SR Ca^{2+} depletion is an important factor for spark termination (Terentyev *et al.*, 2002). Therefore, a possible mechanism for spark termination could be that as depletion of the SR Ca^{2+} occurs during a spark, the RyR2s become deactivated from the intra-SR side (Sobie *et al.*, 2002). Rogue RyR2s or small clusters of RyR2s being less sensitive to Ca^{2+} alterations (Williams *et al.*, 2011) could then shape the kinetics of a spark by being reactivated during the tail of a spark. The plateau phase of long sparks could also be explained by re-activation of small array(s) of RyR2s and rogue RyR2s because of their apparent reduced refractoriness (see below). According to Williams *et al.* (2011), spark termination could be explained by the reduction of Ca^{2+} in the jSR (affecting RyR2 Ca^{2+} sensitivity and Ca^{2+} efflux) and also by the RyR2 cooperativity (a stochastic version of coupled gating).

However, there are several counter-examples that raise important questions. In our experiments, spark restitution can be longer than blink recovery - as much as 3 times in rabbit (Brochet *et al.*, 2005; Brochet *et al.*, 2011). Complex Ca^{2+} spark restitution can also arise when RyR2 behavior is changed (Cheng *et al.*, 1993) and spark termination and refractoriness may also be affected by "adaptation" of the RyR2s (Gyorke and Fill, 1993; Valdivia *et al.*, 1995). Phosphorylation of RyR2s can depend on PKA and CaMKII and these changes may occur dynamically and affect termination and refractoriness (Ramay *et al.*, 2011). Recent experiments also show that Ca^{2+} spark rate can change when RyR2s are oxidized or nitrosylated (Fauconnier *et al.*, 2010; Prosser *et al.*, 2011). Thus refractoriness of local CICR and of Ca^{2+} sparks will vary as diverse spatial, sensitivity, chemical and triggering features of the CRU may change - including those of rogue RyR2s or small arrays of RyR2s (Brochet *et al.*, 2005; Brochet *et al.*, 2011). In this manner repetitive QCR events may be observed during long sparks (Brochet *et al.*, 2011) and the kinetics of restitution may be dynamic and complex.

How many RyR2sare activated during a spark?

The existence of QCR events suggest that there may be diversity of SR Ca^{2+} release events and that, in addition to Ca^{2+} sparks which are the classic SR Ca^{2+} release event at the 20,000 CRUs per cell, there is an additional population of smaller events with diverse kinetics. As noted above, new imaging approaches that include EM and super-resolution imaging suggest that in addition to the "classic" CRU (Franzini-Armstrong *et al.*, 1999), there are

additional organization themes in the CRU (Baddeley *et al.*, 2009; Hayashi *et al.*, 2009). These themes include RyR2 arrays that are spread out as well as those with missing elements. How rogue RyR2s contribute to QCR and normal Ca^{2+} sparks must still be investigated by those of us examining Ca^{2+} release by all experimental means and by mathematical modeling.

This brings up the question of how many RyR2s open to produce a spark. With the initial Sobie model of the Ca^{2+} spark (Sobie *et al.*, 2002), a very broad range of RyR2s could underlie a Ca^{2+} spark (e.g. 10–100). In order to better characterize the quantitative nature of Ca^{2+} sparks, Williams *et al.* (2011) have recently investigated how Ca^{2+} sparks may arise, how individual RyR2s within the CRU may open and fail to activate a Ca^{2+} spark and how Ca^{2+} sparks terminate. This model was based on the latest set of information characterizing RyR2 behavior and information on cardiac ion channels and ion transporters. As with the earlier Sobie model, a wide range of RyR2s may contribute to the Ca^{2+} spark but the character of the "Ca^{2+} leak" was better shown with the Williams model. Depending on the spatial geometry, the sensitivity of the RyR2s and the dynamics of the signal regulation, this modeling may help us understand QCR and their dynamics.

In conclusion, our understanding of RyR2 geometry in the CRU (with both tight clusters and loose clusters and with rogue RyR2s) provides an important background. It complements new information on the dynamic modulation of RyR2 sensitivity. Together they provide a background that will enable us to account for QCR events and organize experiments to investigate how QCRs contribute to Ca^{2+} sparks, normal Ca^{2+} signaling and pathological behavior.

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Abbreviations

AP	action potential
CICR	Ca^{2+}-induced Ca^{2+} release
CRU	Ca^{2+} release unit
EC coupling	excitation-contraction coupling
ER	endoplasmic reticulum
FWHM	full-width at half maximum
LCC	L-type Ca^{2+} channel
QCD	quarky SR Ca^{2+} depletion
QCR	quarky SR Ca^{2+} release
RyR2	type 2 ryanodine receptor
SR	sarcoplasmic reticulum

fSR	free SR
jSR	junctional SR
SERCA2a	SR/ER Ca^{2+} ATPase2a
TT	T-tubule

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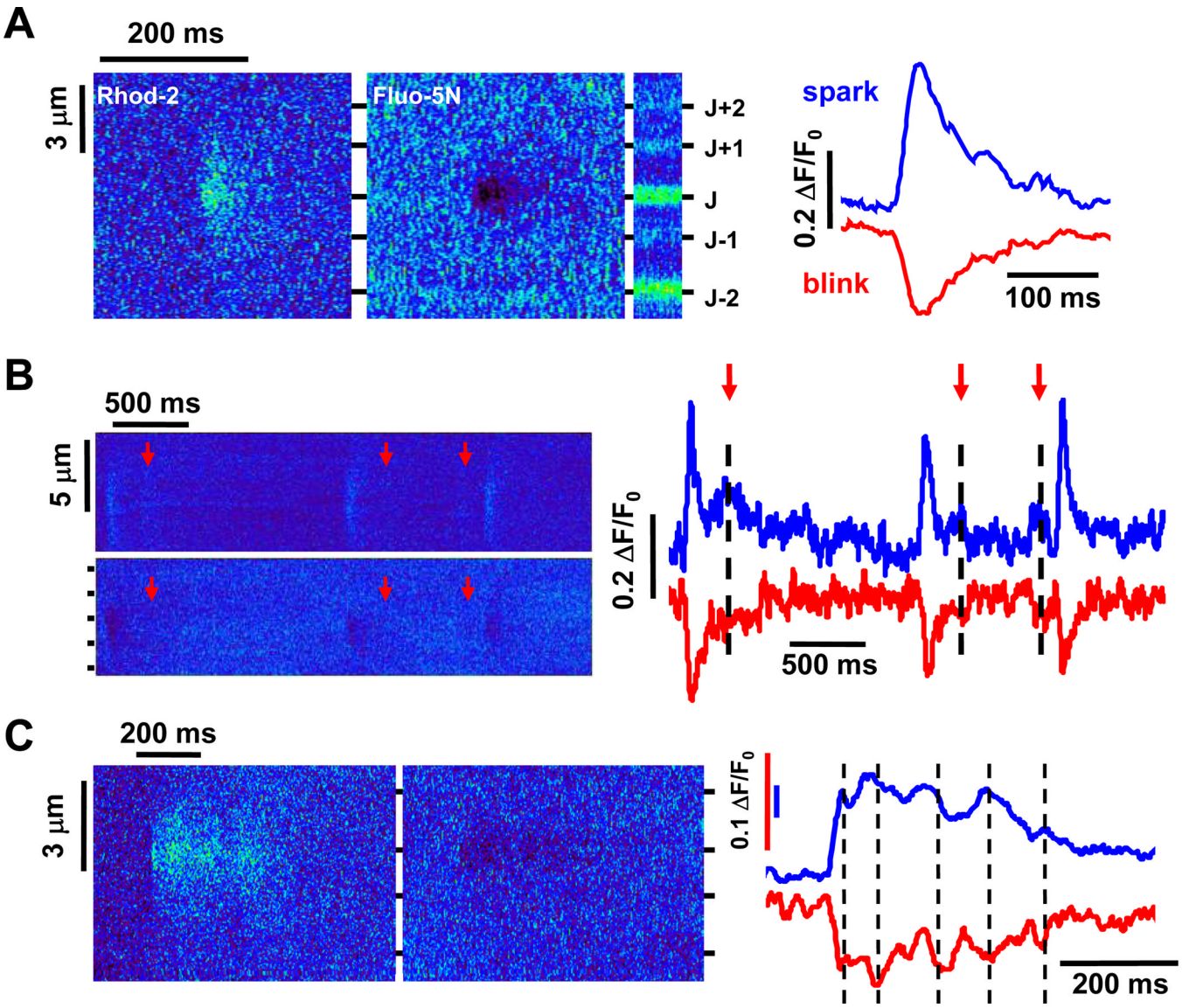

Fig 1. Examples of different elementary Ca^{2+} release events

A. Spark-blink pair. Linescan images of a Ca^{2+} spark (left panel) and its companion Ca^{2+} blink (mid left panel) after background subtraction with the corresponding time courses (right)in an intact rabbit ventricular myocyte. The enrichment of the fluo-5N dye in the junctional sarcoplasmic reticulum (jSR)can be seen on the unsubtracted fluo-5N image (mid-right). The spark-blink pair is centered on the jSR band identified as "J". **B.** Quarky SR Ca^{2+} release (QCR) events. Succession of Ca^{2+} sparks and QCR events on the same jSR (top left) and their companion Ca^{2+} blinks and quarky SR Ca^{2+} depletion (QCD) events (bottom left),with the corresponding time courses (right). The arrows indicate the position of QCR and QCD events on the images and time course plots. **C.** Long spark-blink pair. An example of a long spark (left) and long blink (middle), with the corresponding time courses (right). The dashed lines on the traces show the correspondence between bumps on the spark and dips on the blink.

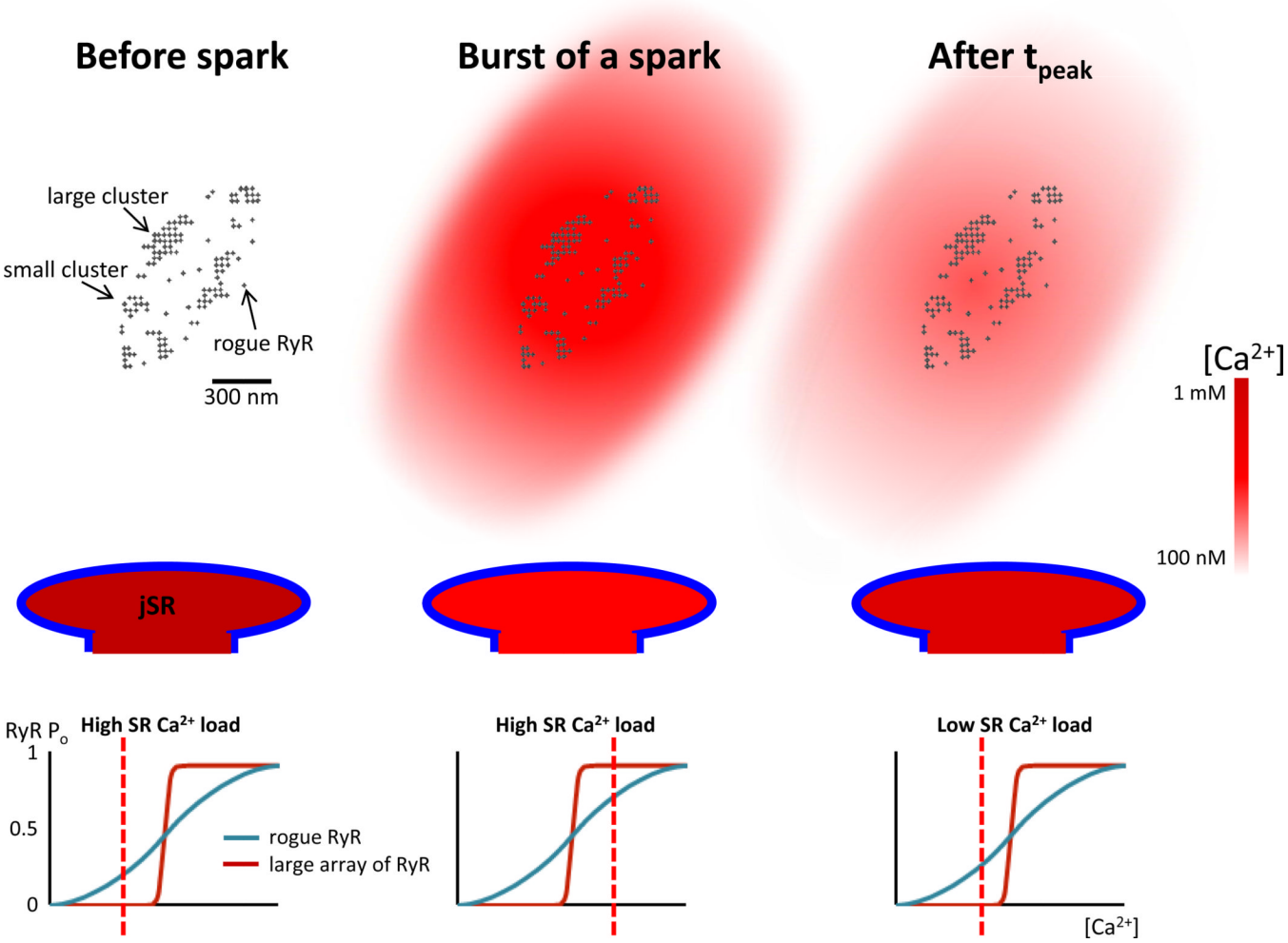

Fig 2. Chronology of the events happening during the time course of a Ca^{2+} spark

Organization of the different clusters of type 2 ryanodine receptors (RyR2s) at a jSR (top). Before the burst of a Ca^{2+} spark, all the RyR2s are in a closed state (top left column). The jSR is fully loaded of Ca^{2+} (mid left column) and the open probability of RyR2s is very low for large array and below 0.5 for rogue RyR2s (bottom left column). During the burst of a Ca^{2+} spark, the increase in cytosolic Ca^{2+} activates the large array of RyR2s (open probability very high) and most of the small arrays or rogue RyR2s (open probability superior at 0.5, bottom middle column). The $[Ca^{2+}]$ at the jSR reaches its nadir (mid middle column) whereas the $[Ca^{2+}]$ in the cytosol attains its peak (top middle column). Once the Ca^{2+} blink has reached its nadir, the low SR $[Ca^{2+}]$ has shifted the activation curves of the large array and rogue RyR2s to the right. The open probability of RyR2s is then very low for large array and below 0.5 for rogue RyR2s (bottom right column). Therefore, only some small arrays or rogue RyR2s will be activated during the tail of a spark (the large array also become refractory). The $[Ca^{2+}]$ in the SR will then increase (mid right column) at the same time as $[Ca^{2+}]$ in the cytosol will diminish (top right column).