

# Quenching the spark: Termination of CICR in the submicroscopic space of the dyad

Mark B. Cannell and Cherrie H.T. Kong

School of Physiology, Pharmacology, and Neuroscience, University of Bristol, Bristol, England, UK

## Introduction

Cardiac excitation–contraction coupling is a transduction cascade that results in muscle contraction and relaxation. In ventricular myocytes, the arrival of an action potential activates sarcolemmal L-type  $\text{Ca}^{2+}$  channels, and the subsequent inward  $\text{Ca}^{2+}$  current, in turn, activates several RyRs in the SR membrane (the  $\text{Ca}^{2+}$  release unit [CRU]). Activation of a CRU causes more  $\text{Ca}^{2+}$  to be released into the local cytoplasm in a process called CICR (Fabiato, 1983) and is observed as a  $\text{Ca}^{2+}$  spark (Cannell et al., 1994). The spatiotemporal summation of these elementary events forms a cell-wide  $\text{Ca}^{2+}$  transient that enables cross-bridge cycling. The rise in cytosolic  $\text{Ca}^{2+}$  is short-lived, as removal mechanisms such as the  $\text{Na}^+$ – $\text{Ca}^{2+}$  exchange and SR  $\text{Ca}^{2+}$  ATPase (SERCA) restore  $\text{Ca}^{2+}$  back to resting conditions, once SR  $\text{Ca}^{2+}$  release stops by one or more mechanisms whose relative contributions remain unclear (Hinch, 2004; Stern and Cheng, 2004), but, as we discuss below, one mechanism—induction decay—can by itself explain the termination of CICR.

## As an amplifier, CICR needs local control

CICR amplifies a small trigger  $\text{Ca}^{2+}$  flux by approximately one order of magnitude by inducing  $\text{Ca}^{2+}$  release from the SR. Although the trigger is provided (mostly) by L-type  $\text{Ca}^{2+}$  channel gating during the action potential, SR  $\text{Ca}^{2+}$  release is mediated by  $\text{Ca}^{2+}$ -dependent gating of RyRs that are the SR  $\text{Ca}^{2+}$  release channels. As soon as it became possible to measure  $\text{Ca}^{2+}$  levels inside voltage-clamped cardiac cells, it became apparent that regenerative CICR never escapes tight control by the timing and amplitude of the trigger  $\text{Ca}^{2+}$  influx, although the RyRs should be regenerative activated by their own  $\text{Ca}^{2+}$  release (as both sources feed the adjacent cytoplasm or the “common pool”; Cannell et al., 1987). A mathematical analysis by Stern (1992) showed that common pool CICR models should operate in an “all or none” fashion at realistic flux amplification levels, which was clearly at odds with the graded SR  $\text{Ca}^{2+}$

release seen in numerous single-cell voltage-clamp experiments (Barceñas-Ruiz and Wier, 1987; Cannell et al., 1987). The solution to this problem was provided by “local control” (Stern, 1992, 1999; Cannell et al., 1995; Soeller and Cannell, 2004), wherein small groups of RyRs and L-type  $\text{Ca}^{2+}$  channels form an autonomous CRU in the microanatomical dyad structure (Franzini-Armstrong et al., 1998). The physical separation of CRUs by several hundred nanometers prevents cell-wide regenerative behavior and gives rise to microscopic packets of  $\text{Ca}^{2+}$  release, which were first detected in the form of  $\text{Ca}^{2+}$  “sparks” (Cheng et al., 1993). Thus, activation of one or a small number of RyRs within a CRU leads to rapid recruitment of the adjacent RyRs within that CRU to produce a  $\text{Ca}^{2+}$  spark, but RyRs in adjacent CRUs are not normally activated because of the diffusion and buffering of  $\text{Ca}^{2+}$  outside the source dyad. Graded cell-wide  $\text{Ca}^{2+}$  release is then provided by the time- and trigger-dependent recruitment of  $\text{Ca}^{2+}$  sparks, whose amplitude depends on SR  $\text{Ca}^{2+}$  levels (Cannell et al., 1995; Soeller and Cannell, 2004).

However, the regenerative problem inherent in CICR was not solved at the scale of the CRU by the discovery of  $\text{Ca}^{2+}$  sparks. Once a CRU is activated, a  $\text{Ca}^{2+}$  spark should still progress independently of the trigger because of regenerative CICR within the dyad junction itself (Cannell et al., 1987, 1995). Put another way, the dyad space should (essentially) recapitulate the original common pool problem. Mathematical analysis has shown that most proposed mechanisms can contribute to stability (Hinch, 2004), but which is most important or key?

To date, the mechanisms responsible for the control of SR release termination remain unclear, although evidence for several mechanisms that may contribute to RyR closure have been obtained: (1) time-dependent inactivation and/or “adaptation” of the RyR channel (Györke and Fill, 1993; Zahradníková and Zahradník, 1996; Vélez et al., 1997); (2) stochastic attrition, which describes the probabilistic event that all ( $n$ ) RyR channels within a CRU close at the same time to allow the

Correspondence to Mark B. Cannell: mark.cannell@bristol.ac.uk

Abbreviations used: CRU,  $\text{Ca}^{2+}$  release unit; CSQ, calsequestrin; FKBP, FK506-binding protein; HRC, histidine-rich  $\text{Ca}^{2+}$ -binding protein; jSR, junctional SR;  $P_o$ , open probability; PSF, point spread function; SERCA, SR  $\text{Ca}^{2+}$  ATPase.

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local  $\text{Ca}^{2+}$  to dissipate and thus terminate regenerative CICR (Stern, 1992); (3) allosteric coupling between RyRs so that spontaneous closure of one RyR promotes closure of the others (Stern, 1992; Marx et al., 2001; Sobie et al., 2002); (4) SR  $\text{Ca}^{2+}$ -dependent RyR gating changes caused by the presence of a RyR luminal  $\text{Ca}^{2+}$  sensor either on the RyR itself (Györke and Györke, 1998; Ching et al., 2000) or via an accessory protein such as calsequestrin (CSQ; Qin et al., 2008); and (5) “induction decay” (Laver et al., 2013) or “pernicious attrition” (Gillespie and Fill, 2013), wherein a decreasing RyR release flux leads to local cytoplasmic  $\text{Ca}^{2+}$  levels becoming insufficient to maintain CICR. All of these mechanisms with the exception of induction decay have been discussed in previous focused reviews (Fill and Cello, 2002; Stern and Cheng, 2004; Cannell and Kong, 2012), so this perspective will not exhaustively examine this literature except to raise problems in their sufficiency for CICR termination.

#### Control via the SR lumen

$\text{Ca}^{2+}$  in the lumen of the SR is highly buffered, and CSQ appears to be able to explain most of the measured buffering power (Shannon and Bers, 1997). In addition to this important role, CSQ may also directly modulate RyR gating, an idea supported by the  $\text{Ca}^{2+}$  handling abnormalities associated with CSQ mutants and CSQ expression changes (Terentyev et al., 2003; Knollmann et al., 2006). In addition, histidine-rich  $\text{Ca}^{2+}$ -binding proteins (HRCs) are also present in the SR and may modulate SERCA  $\text{Ca}^{2+}$  uptake as well as RyR gating (Arvanitis et al., 2011). The amount of HRC present in the SR is uncertain but seems capable of supplanting  $\text{Ca}^{2+}$  binding in CSQ-knockout mice (Murphy et al., 2011). However, most (if not all)  $\text{Ca}^{2+}$  transport/balance models have focused on CSQ as the principal SR  $\text{Ca}^{2+}$  buffer and have not included HRCs. Finally, it should be noted that SERCA also buffers  $\text{Ca}^{2+}$  in the lumen of the SR, and this buffer can modify  $\text{Ca}^{2+}$  cycling (Higgins et al., 2006).

Eventual termination of CICR would be assured if the SR ran out of buffered  $\text{Ca}^{2+}$  (Fig. 1 A); however, measurements of SR content using caffeine as a probe of releasable  $\text{Ca}^{2+}$  suggested that less than 50% of the SR  $\text{Ca}^{2+}$  content was released in a single twitch (Bassani et al., 1993). Thus, extensive SR-wide  $\text{Ca}^{2+}$  depletion is unlikely to explain release termination. Because CICR is a local control phenomenon in the dyad (Cannell et al., 1995; Stern et al., 1999), attention has turned naturally to evaluating  $\text{Ca}^{2+}$  levels in the junctional SR (jSR). Measurements with low-affinity  $\text{Ca}^{2+}$  indicators trapped within the SR also showed that jSR  $\text{Ca}^{2+}$  depletion was far from complete (Shannon et al., 2003; Brochet et al., 2005), and it was suggested that depletion by itself could not explain CICR termination (Sobie et al., 2002). However, a more moderate depletion could

(possibly) be augmented by SR luminal control of RyR gating (Fig. 1 B).

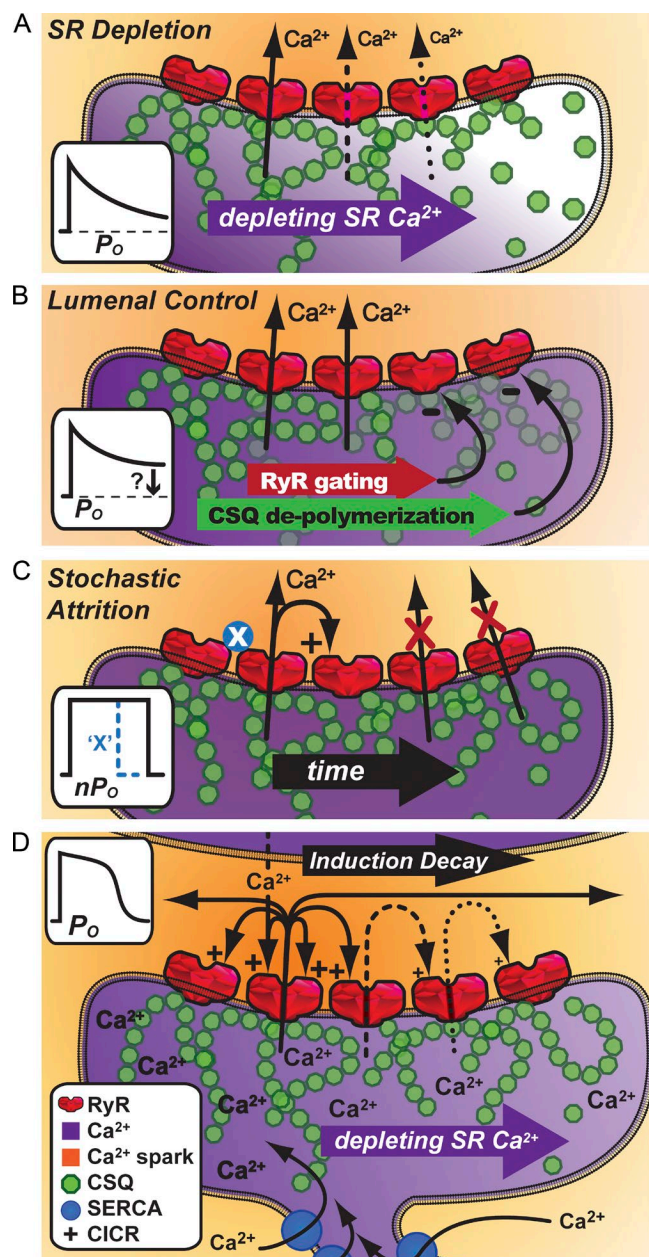
RyR gating appears to be sensitive to the level of  $\text{Ca}^{2+}$  in the SR lumen (Sitsapesan and Williams, 1994; Lukyanenko et al., 1996; Györke and Györke, 1998). Varying luminal  $\text{Ca}^{2+}$  over the likely physiological range (0.5–2 mM) alters RyR open probability ( $P_O$ ) approximately twofold (Györke and Györke, 1998) and, although weaker than cytoplasmic regulation, could be important in adjusting CICR gain. A large part of RyR luminal  $\text{Ca}^{2+}$  sensitivity may be related to CSQ binding, because when CSQ is stripped from the RyR complex, RyR luminal  $\text{Ca}^{2+}$  sensitivity is reduced by a factor of  $\sim 2$ , as is the maximum  $P_O$  (Ching et al., 2000; Qin et al., 2008). Such a moderate change in RyR gating would not seem capable of terminating SR  $\text{Ca}^{2+}$  release without augmentation by additional mechanisms. Our modeling suggests that such luminal control is only a weak modifier of  $\text{Ca}^{2+}$  release during  $\text{Ca}^{2+}$  sparks (Cannell et al., 2013) and produces effects that are hard to distinguish from modifiers of the cytoplasmic  $\text{Ca}^{2+}$  sensitivity of RyRs.

#### RyR inactivation/adaptation

Time-dependent inactivation and/or “adaptation” (Györke and Fill, 1993) may be seen under some conditions, and many models of CICR include RyR inactivation to achieve stability. However, adaptation appears to be too slow (in the order of  $\sim 100$  ms; Valdivia et al., 1995) to be responsible for  $\text{Ca}^{2+}$  spark termination and, on the  $\sim 30$  ms timescale of the  $\text{Ca}^{2+}$  spark, significant adaptation/inactivation is not seen (Zahradníková et al., 1999). Furthermore, direct evidence against adaptation as a primary termination mechanism was provided by local  $\text{Ca}^{2+}$  release measurements (Sham et al., 1998). However, this does not mean that adaptation-type mechanisms are incapable of adding some modulation to other CICR termination (and activation) processes. In connection with this point, it has been suggested that resting  $\text{Ca}^{2+}$  spark rate can increase slowly during rest with  $\text{Ca}^{2+}$  influx blocked and no change in SR  $\text{Ca}^{2+}$  load (Satoh et al., 1997), a phenomenon that would be compatible with some weak, time-dependent processes. In addition, RyRs may undergo modal gating behavior with a slow transition between a high availability mode and other states (Zahradníková and Zahradník, 1995). Again, although such gating changes could contribute to longer-term changes in RyR responses, the rate of mode shifting appears to be too slow for this process to play a major role in  $\text{Ca}^{2+}$  spark termination.

#### Stochastic attrition

Stochastic attrition (Fig. 1 C) also appears to be too slow to explain normal  $\text{Ca}^{2+}$  spark termination for typical RyR open times,  $P_O$ , and likely the number of RyRs in a CRU (Stern and Cheng, 2004; Cannell and Kong,



**Figure 1. Possible CICR termination mechanisms.** (A) SR  $\text{Ca}^{2+}$  depletion. A reduction in SR  $\text{Ca}^{2+}$  levels will reduce release flux regardless of RyR gating (as described by their open probability,  $P_0$ ). However, fateful termination by this mechanism alone is problematic, because the jSR lumen is continually refilled from the rest of the SR. (B) SR luminal control of RyR gating may be modulated either by a direct effect on the RyR itself (red) and/or via an accessory protein such as CSQ (green). However, the extent to which these mechanisms could reduce RyR  $P_0$  sufficiently to terminate release is unclear. (C) Stochastic attrition. If all RyRs close simultaneously, then the release flux is terminated. However, it is unlikely that this will occur within the timescale of a  $\text{Ca}^{2+}$  spark. Stochastic attrition could be accelerated by coupled gating between RyRs, either by direct contact or by a protein linker (X). (D) Induction decay. After CRU activation, jSR  $\text{Ca}^{2+}$  levels decline which results in a decreasing release flux. The local cytoplasmic  $\text{Ca}^{2+}$  level is proportional to the release flux, and this is transduced via the steep  $\text{Ca}^{2+}$  dependence of

2012). However, recent super-resolution data indicate that the number of RyRs in each junctional cluster may be lower than originally inferred from junctional area and the assumption of tight RyR packing within circular clusters (Baddeley et al., 2009; Hou et al., 2015). Previous electron microscopy and confocal imaging studies suggested that up to several hundred RyRs might form a functional CRU in each junction (Franzini-Armstrong et al., 1999; Soeller et al., 2007), but the organization of the RyRs in the CRU is highly variable and occupies an average area that would correspond to 40 to 60 RyRs per CRU if tightly packed (Hou et al., 2015). Because RyRs may not be tight packed (see Allosteric coupling), the number of RyRs inferred from junctional image area should probably be reduced by ~30% to 50% to give ~30 to 40 RyRs in each functional CRU. From this, we can calculate that the maximum release flux would be ~7 pA from a single channel current of ~0.4 pA (Gillespie and Fill, 2008) and peak  $P_0$  of 0.5 (Cannell et al., 2013), which is close to that estimated from  $\text{Ca}^{2+}$  spark model fitting (Soeller and Cannell, 2002). However, with an open time of ~2 ms, the time constant of stochastic attrition would still be too long for attrition to play a key role unless  $P_0$  is reduced to <0.1 (Stern and Cheng, 2004), which seems unlikely for junctional  $\text{Ca}^{2+}$  levels >10  $\mu\text{M}$  that arise from a release flux >0.2 pA (Soeller and Cannell, 1997).

A key defining feature of the stochastic attrition mechanism is the near-simultaneous closure of all currently open RyRs in the CRU to allow local cytoplasmic  $\text{Ca}^{2+}$  to decline to a level that does not reopen them. Stochastic attrition should be associated with a rather abrupt cessation of release flux, but our detailed release flux calculations suggested a rather smooth decrease in release flux during the  $\text{Ca}^{2+}$  spark (Soeller and Cannell, 2002; Kong et al., 2013), although this is not a very strong argument against stochastic attrition in the face of uncertainties caused by noise and microscope blurring (see Reanalysis of SR  $\text{Ca}^{2+}$  depletion signals).

For CICR to stop fatefully under stochastic attrition, local  $\text{Ca}^{2+}$  levels in the dyad must decline to a level that prevents any RyRs from reopening. It takes local  $\text{Ca}^{2+}$  ~5 ms to decrease to near mean cytoplasmic levels after SR release stops (Soeller and Cannell, 1997). If this is much shorter than the mean closed time divided by the number of RyRs, CICR would not be able to reignite the  $\text{Ca}^{2+}$  spark, and release would be terminated. Therefore, although stochastic attrition might not be an initiating event for termination of CICR, full termination still requires that the RyR closed time divided by the number of RyRs in a cluster be >5 ms. For a cluster of ~35 RyRs,

the RyR closed time. As the closed time becomes longer, it becomes less and less likely for an RyR to reopen to provide the flux and local  $\text{Ca}^{2+}$  levels required to continue CICR.



this would imply a RyR closed time of more than  $\sim 165$  ms, and this is seen at a cytoplasmic  $\text{Ca}^{2+}$  concentration of  $<4\text{--}40\text{ }\mu\text{M}$  (depending on species; Cannell et al., 2013). Such levels are likely to be achieved within  $\sim 5$  ms of CRU closure, so stochastic attrition by itself could finally terminate CICR, although some other mechanism may be responsible for initially reducing  $n\text{P}_\text{O}$  so that stochastic attrition can occur in a timely manner.

Timescale-based arguments against stochastic attrition being the mechanism for  $\text{Ca}^{2+}$  spark termination do not apply when the availability of RyRs is reduced with tetracaine, because long-lasting  $\text{Ca}^{2+}$  sparks can occur (Zima et al., 2008a). This is associated with an apparently steady level of SR  $\text{Ca}^{2+}$ , and so SR release termination cannot be caused by changes in luminal  $\text{Ca}^{2+}$  (or a luminal SR  $\text{Ca}^{2+}$ -sensing site) in these conditions (Zima et al., 2008b). However, we suggest that the termination of such long-lasting release events (lasting  $\sim 300$  ms or more) is compatible with the stochastic attrition mechanism.

#### Allosteric coupling

The equation for the time constant for stochastic attrition (Stern and Cheng, 2004) depends on the assumption of independent RyR gating, but it has been suggested that RyR gating might not be independent (Fig. 1 C, "X"). When RyRs are reconstituted in bilayers, RyRs can show coupled gating (Marx et al., 2001), and RyRs are closely packed in the junctional space (Franzini-Armstrong et al., 1999), suggesting the possibility of allosteric interactions between RyRs. Such allosteric coupling could produce positive cooperativity, which would cause a CRU to behave as if there were fewer RyRs in the cluster (Stern, 1992; Sobie et al., 2002; Stern and Cheng, 2004; and in the limit of very strong coupling causes the cluster to gate as one). Although this is a viable mechanism to produce reliable  $\text{Ca}^{2+}$  sparks and spark termination (Stern and Cheng, 2004; Groff and Smith, 2008), how possible *physical* interactions (distinct from the effects mediated by changes in  $\text{Ca}^{2+}$ ) might occur is unclear. FK506-binding protein (FKBP) was initially identified as a protein modifier of RyR1 interactions (Marx et al., 1998), but its possible role in coupled RyR gating is controversial, with conflicting evidence for roles in determining  $\text{Ca}^{2+}$  spark frequency and properties (Guo et al., 2010 and references therein).

A protein that acts as a linker between RyR tetramers might be expected to have 1:1 or 1:2 stoichiometry with RyR, but  $<20\%$  of RyRs have FKBP12.6 (the isoform that appears to modify RyR gating) bound, although RyR binds nearly all the FKBP12.6 in the cell (Guo et al., 2010). Allosteric interactions require RyR to be very closely apposed, if not actually touching. Recent high-resolution tomographic data suggest that RyRs in cardiac dyads do not exhibit a regular geometric organization, with only  $\sim 50\%$  actually touching each other

(Asghari et al., 2014). This result, although compatible with the low fraction of RyR actually having FKBP bound, would place an important limit on the extent to which RyR allosteric interactions (and consequent increase in likelihood of stochastic attrition) can help fateful  $\text{Ca}^{2+}$  spark termination by attrition (Hinch, 2004). This conclusion is supported by experiments in FKBP12.6-null mice, which show only modest increases in spontaneous  $\text{Ca}^{2+}$  spark frequency and duration (Xin et al., 2002). Recently, high-resolution electron micrographs of purified RyRs appear to show that there may be some preferred regions of RyR interaction that can cause them to form dimers (Cabra et al., 2016), but this interaction is likely weak, because most RyRs did not dimerize or form higher number assemblies. We suggest that if coupled gating via physical interactions occurs, it is neither central to the spark termination problem nor a major modifier of  $\text{Ca}^{2+}$  release during  $\text{Ca}^{2+}$  sparks.

#### Induction decay

None of the aforementioned mechanisms, in isolation, appears to be capable of providing a sufficient explanation for  $\text{Ca}^{2+}$  spark termination (Stern and Cheng, 2004). However, most prior models for CICR did not include realistic geometry for the RyRs in the dyad or an accurate description for RyR gating under physiological conditions. These shortcomings were addressed in a new induction decay model (Fig. 1 D), which included a simplified RyR gating model (based on actual RyR gating measured in planar lipid bilayers) as well as dyad geometry (Laver et al., 2013). The mechanism of  $\text{Ca}^{2+}$  spark termination that appeared as an emergent property of the model was called induction decay because it reflected the gradual loss of the regenerative capacity (or gain) within  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. In the model, a gradual decline in local  $\text{Ca}^{2+}$  caused by a decreasing open RyR  $\text{Ca}^{2+}$  flux resulted in a steep increase in the closed time of adjacent RyRs, so it became increasingly unlikely for CICR to continue (as also shown in the mathematical analysis of Hinch [2004]). The decline in RyR release flux was entirely caused by local  $\text{Ca}^{2+}$  depletion in the jSR, which refilled, once release was finished, from the network SR. Importantly, the model also explained the time course of  $\text{Ca}^{2+}$  spark restitution described by Sobie et al. (2005) without additional free parameters.

That a decreasing RyR flux could affect SR release was shown directly in cotemporaneous experiments using RyR permeation blockers (Guo et al., 2012). Although these observations were subsequently incorporated into a termination mechanism called pernicious attrition (Gillespie and Fill, 2013), the idea of induction decay is central to both the computational and conceptual models. The strength of the computer model (Cannell et al., 2013) resides in its ability to show that the measured  $\text{Ca}^{2+}$  dependence of RyR closed times is sufficient

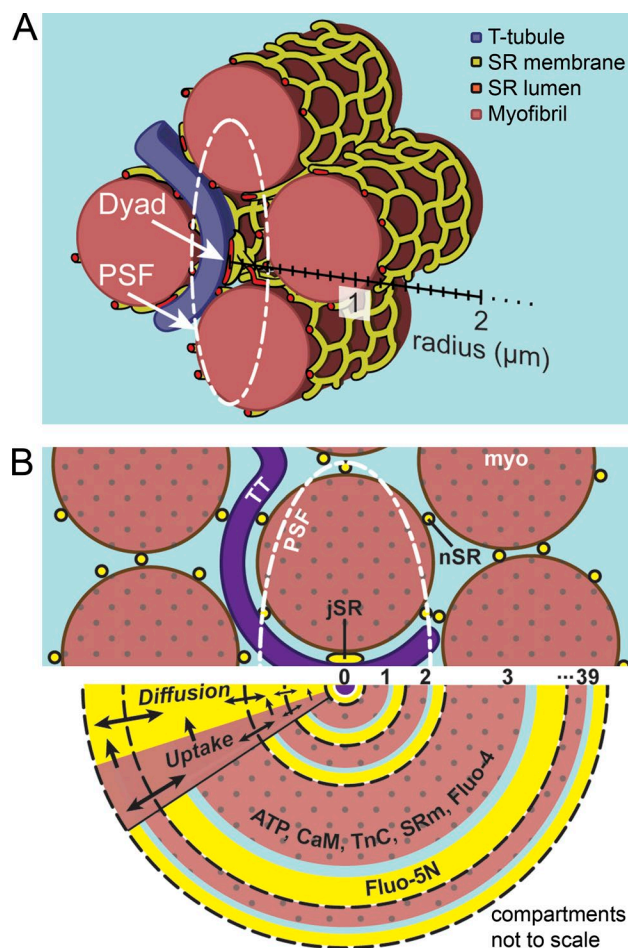
to terminate CICR, as well as reproduce other effects such as  $\text{Ca}^{2+}$  spark refractoriness. However, it remains unclear whether any SR load-dependent RyR gating effects might also be present to modulate induction decay (see above). The coupling of jSR load to the ability to support CICR via the dyad cytoplasmic space provides an effective “use dependence” that was observed by Sham et al. (1998) in “ $\text{Ca}^{2+}$  spike” recordings that give a measure of local release fluxes.

While various alternative models can be tuned to control CICR under a fixed set of conditions (Stern, 1992), the induction decay model produced similar  $\text{Ca}^{2+}$  sparks with variable numbers of RyRs, RyR organization, and RyR  $\text{Ca}^{2+}$  sensitivity, and this remarkable property was caused by the extent of local jSR depletion associated with the CRU. The relative insensitivity to the number of RyRs in the dyad in the induction decay model is unlike models that rely on simple attrition schemes and would be an advantage for variable RyR expression in dyads. Similarly, a significant increase in RyR sensitivity (as seen in sheep RyRs) does not prevent  $\text{Ca}^{2+}$  spark termination, because the jSR was simply depleted to a lower level (Cannell et al., 2013), a feature reminiscent of the behavior of CICR as seen in the presence of RyR gating modifiers (Eisner et al., 2000). A more recent study (Walker et al., 2014) using the geometry and RyR gating used in the original induction decay model showed that it could also mimic the SR  $\text{Ca}^{2+}$  leak–load relationship as seen in intact cells (Zima et al., 2010). In the induction decay model, such effects are mediated by cytoplasmic dependence of RyR opening rate and consequent support of CICR (initiated by a spontaneous RyR opening) rather than a luminal  $[\text{Ca}^{2+}]$  effect by itself. It is important to note that in the induction decay model, the number of open RyRs gradually decreases, unlike the abrupt simultaneous closure required for stochastic attrition. Of course, once the number of open RyRs becomes small enough, stochastic attrition may finish the induction decay process (Hinch, 2004), but simultaneous closure of multiple RyRs is not needed and does not usually occur.

Perhaps unexpectedly, our induction decay simulations also showed that the standard deviation of  $\text{Ca}^{2+}$  spark durations ( $\sim 10\%$  of the mean; Table 1 in Cannell et al., 2013) was smaller than might be expected for a purely stochastic closing process. A part of this behavior can be explained by RyR gating being supplied with an effective memory of the prior RyR gating pattern because of the coupling of prior RyR openings to the level of  $\text{Ca}^{2+}$  in the jSR that, in turn, affects RyR gating (via  $\text{Ca}^{2+}$  the dyadic space). This behavior also manifests a type of “allosteric coupling” between RyRs, mediated not by direct RyR contact but via  $\text{Ca}^{2+}$  flux–dependent cross-talk.

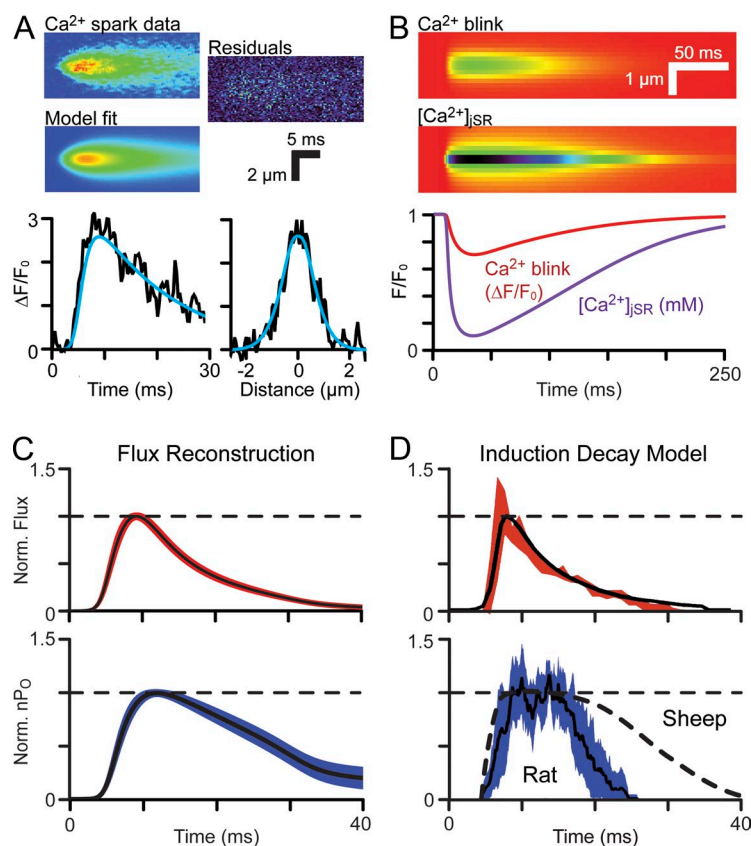
#### SR depletion as another local control phenomenon

Local depletion of the jSR is required for induction decay, and the depth of depletion (to  $\sim 10\%$  of the orig-



**Figure 2. A method for calculating jSR release fluxes from spark records.** (A) A simplified diagram illustrating the relative sizes of the T-tubules, jSR, network SR (nSR), myofilaments (myo), and a typical microscope PSF that blurs the  $\text{Ca}^{2+}$  signals. (B) By using spherical geometry for the model, the computational complexity is greatly reduced while still retaining the volume fractions and concentrations of each component that contribute to  $\text{Ca}^{2+}$  fluxes. Effectively, the spatial geometries of each component are spread over each computational compartment, making transport from the jSR isotropic at the length scale of interest (200 nm). The center element is dedicated to the dyadic space (including the jSR with CSQ; Kong et al., 2013), and all other compartments include reaction and diffusion fluxes in the cytosol and SR as indicated in the bottom part of the figure. Having calculated the spatial Fluo-4 and Fluo-5N signals, these are blurred by convolution with the microscope PSF, and the resulting signal is fitted to typical  $\text{Ca}^{2+}$  sparks by altering the time course of the jSR permeability change basis function (Kong et al., 2013). Because large numbers of simulations are performed to allow the release waveform to converge, efficiency in solving the discretized transport equations after microscope blurring is important.

inal level) is much larger than suggested by previous experimental studies. Using caffeine to probe the total SR  $\text{Ca}^{2+}$  content, it has been estimated that the SR releases 17% to 53% of its content (Bassani et al., 1993; Delbridge et al., 1996; Díaz et al., 1997). A similar estimate ( $\sim 50\%$ ) is provided by  $\text{Ca}^{2+}$  imaging with SR-



**Figure 3. Estimating jSR  $\text{Ca}^{2+}$  depletion and RyR gating time course by flux reconstructions.** (A) The 3-D model of  $\text{Ca}^{2+}$  reactions, diffusion, and microscope blurring shown in Fig. 2 generates a  $\text{Ca}^{2+}$  spark record, which is fitted to experimental data by varying a basis function for the jSR permeability time course (Kong et al., 2013). (B) Although not fitted, the model can also calculate the corresponding SR  $\text{Ca}^{2+}$  depletion signals, which are similar to those recorded experimentally (Brochet et al., 2005). (C) From the calculated release flux (red) and the jSR  $\text{Ca}^{2+}$  levels, the jSR permeability time course was derived (normalized flux in red and normalized  $n\text{Po}$  [number of open channels] in blue). (D) Normalized release flux (red) and permeability (blue) changes from the induction decay model of Cannell et al. (2013). Note the concordance of flux estimates by both models, although the permeability time course is dependent on species-dependent RyR  $\text{Ca}^{2+}$  sensitivity.

loaded Fluo-5N (Shannon et al., 2003; Zima et al., 2010; Picht et al., 2011). Such moderate depletion might seem to be a problem for the induction decay mechanism. However, we suggest the local jSR is more deeply depleted than the latter imaging studies suggest.  $\text{Ca}^{2+}$  sparks that were repeatedly activated from the same site showed that  $\text{Ca}^{2+}$  spark amplitude decreased with decreasing interval between activations ( $\Delta t$ ), and at  $t$  short  $\Delta t$  ( $\sim 50$  ms),  $\text{Ca}^{2+}$  spark amplitude was only  $\sim 10\%$  of the initial  $\text{Ca}^{2+}$  spark amplitude (Sobie et al., 2005), suggesting that local SR  $\text{Ca}^{2+}$  may be similarly reduced. Importantly, the restitution of  $\text{Ca}^{2+}$  spark amplitude in the latter study was reproduced by the induction decay model, further strengthening the idea of significant local SR depletion, although this would not rule out a lesser depletion augmented by some other form of luminal control.

#### Reanalysis of SR $\text{Ca}^{2+}$ depletion signals

To further examine the possibility that local jSR depletion may be deeper than suggested by fluorescence measurements, we performed a detailed  $\text{Ca}^{2+}$  spark model fitting exercise (Kong et al., 2013), similar to an earlier analysis of  $\text{Ca}^{2+}$  spark flux by a reconstruction method (Soeller and Cannell, 2002). We constructed a spherical reaction–diffusion model centered on a single dyad (Fig. 2). Cellular structures and associated  $\text{Ca}^{2+}$  buffers were homogeneously distributed over each model com-

partment (Fig. 2 B), and the calculated fluorescence signals at all model spatiotemporal coordinates were then convolved with a model of the measured microscope point spread function (PSF) to simulate experimental  $\text{Ca}^{2+}$  spark recordings. Importantly, the model confocal PSF was not assumed to be diffraction limited but matched to that observed in live-cell experiments. The jSR was given a volume and buffering power consistent with other models, and high-quality  $\text{Ca}^{2+}$  spark records were fitted by modifying a release flux basis function. This model accurately fitted  $\text{Ca}^{2+}$  spark data (Fig. 3 A) both temporally and spatially (Fig. 3 A, bottom) and produced a reasonable “ $\text{Ca}^{2+}$  blink” depletion signal (Fig. 3 B), although the actual level of SR  $\text{Ca}^{2+}$  depletion was lower than the fluorescence record ( $F/F_0$ ) might suggest. This difference arises from the blurring of the Fluo-5N signal, which is more spatially restricted compared with that of a  $\text{Ca}^{2+}$  spark and its nonlinear response to  $\text{Ca}^{2+}$  (Kong et al., 2013). The time to minimum of the induction decay model blink signal ( $\sim 25$  ms) is very similar to that reported in the original work of Brochet et al. (2005) of 24 ms (see also Terentyev et al., 2008), although another study in skinned cells suggested longer times to peak of the  $\text{Ca}^{2+}$  spark and blink ( $\sim 60$  ms; Zima et al., 2008b). The time to nadir depends on the degree of jSR connectivity, jSR buffering, and RyR cytoplasmic  $\text{Ca}^{2+}$  sensitivity, so some variability among experiments should be expected. Nevertheless,



one consistently observed property is that the time to nadir of a  $\text{Ca}^{2+}$  blink is 1.5 to 3 times longer than the time to peak of the associated  $\text{Ca}^{2+}$  spark, and this is reproduced in the computational model (see Fig. 3).

From the deduced release flux and jSR depletion, the apparent RyR gating time course was derived (Fig. 3 C,  $n\text{P}_\text{O}$ ). Two features of this analysis were notable: (1) the release flux appeared to decay monotonically, whereas (2) the jSR permeability declined more slowly. The experimentally constrained model flux was very similar to the mean (stochastic) induction decay model results (Fig. 3 D). The time course of RyR gating differs between models. The decline of RyR permeability is sensitive to the  $\text{Ca}^{2+}$  dependence of RyR closed time, as shown by the more  $\text{Ca}^{2+}$ -sensitive sheep RyRs (Fig. 3 D, dashed line). Despite these changes in gating time course, these models suggest that the decay of release flux is driven mainly by the local  $\text{Ca}^{2+}$  gradient across the jSR membrane rather than the time course of RyR gating per se. The calculated jSR depletion levels are consistent with the depletion required for induction decay.

## Conclusion

The induction decay mechanism provides a self-sufficient explanation for CICR termination. In this mechanism, a decline in jSR  $\text{Ca}^{2+}$  during a  $\text{Ca}^{2+}$  spark is transduced via the steep cytoplasmic  $\text{Ca}^{2+}$ -dependence of RyR gating, and this rapidly increases RyR closed time until CICR cannot be maintained. The other mechanisms described here may be able to modulate induction decay, although further studies will be needed to establish their relative contributions. We suggest that additional modification or modifications of the gain and sensitivity of CICR may be necessary because of the criticality of CICR for cardiac function and, therefore, may need more than one point of control.

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