Spark-Induced Sparks As a Mechanism of Intracellular Calcium Alternans in Cardiac Myocytes

Robert Rovetti, Xiaohua Cui, Alan Garfinkel, James N. Weiss, Zhilin Qu

- <u>Rationale</u>: Intracellular calcium (Ca) alternans has been widely studied in cardiac myocytes and tissue, yet the underlying mechanism remains controversial.
- <u>Objective</u>: In this study, we used computational modeling and simulation to study how randomly occurring Ca sparks interact collectively to result in whole-cell Ca alternans.
- <u>Methods and Results</u>: We developed a spatially distributed intracellular Ca cycling model in which Ca release units (CRUs) are locally coupled by Ca diffusion throughout the myoplasm and sarcoplasmic reticulum (SR) network. Ca sparks occur randomly in the CRU network when periodically paced with a clamped voltage waveform, but Ca alternans develops as the pacing speeds up. Combining computational simulation with theoretical analysis, we show that Ca alternans emerges as a collective behavior of Ca sparks, determined by 3 critical properties of the CRU network from which Ca sparks arise: "randomness" (of Ca spark activation), "refractoriness" (of a CRU after a Ca spark), and "recruitment" (Ca sparks inducing Ca sparks in adjacent CRUs). We also show that the steep nonlinear relationship between fractional SR Ca release and SR Ca load arises naturally as a collective behavior of Ca sparks, and Ca alternans can occur even when SR Ca is held constant.
- <u>Conclusions</u>: We present a general theory for the mechanisms of intracellular Ca alternans, which mechanistically links Ca sparks to whole-cell Ca alternans, and is applicable to Ca alternans in both physiological and pathophysiological conditions. (*Circ Res.* 2010;106:1582-1591.)

Key Words: calcium sparks a calcium alternans a randomness a refractoriness a recruitment

ntracellular calcium (Ca) cycling plays a central role in L cardiac excitation-contraction coupling.^{1,2} Ca alternans, a beat-to-beat alternation in intracellular Ca transient amplitude, is an important factor promoting T-wave alternans and pulsus alternans, markers conferring an increased risk of sudden cardiac death.3 Although Ca alternans has been widely studied in cardiac myocytes,4-13 the underlying mechanism remains controversial. Eisner et al⁴ were the first to propose that Ca alternans could be explained by a steep nonlinear dependence of sarcoplasmic reticulum (SR) Ca release on the diastolic SR Ca load immediately preceding the release (a steep fractional release-load relationship). This mechanism requires that diastolic SR Ca load alternate concomitantly with SR Ca release. Subsequent experimental6,7,9 and theoretical9,14,15 studies have provided evidence supporting this mechanism. However, later experimental studies in rabbit ventricular myocytes by Picht et al¹² and in cat atrial myocytes by Hüser et al13 showed that under some conditions, Ca alternans can be dissociated from the expected alternation in SR Ca content, raising questions about whether the mechanism proposed by Eisner et al is universally valid. Based on their observations, Picht et al suggested that refractoriness of ryanodine receptors (RyRs) might play an important role in frequency-dependent Ca alternans.

In this study, we developed a spatially distributed Ca cycling model to further investigate these issues. The model consists of a quasi-2D array of Ca release units (CRUs) (also called couplons) that are coupled through Ca diffusion in the myoplasm and the SR network, from which Ca sparks, the elementary Ca release events of excitation-contraction coupling,^{16,17} arise. According to present understanding, Ca sparks can be triggered by 3 mechanisms: (1) by opening of one or more L-type Ca channels (LCCs) in a CRU during an action potential, through a process called Ca-induced Ca release (CICR); (2) by spontaneous openings of RyRs, especially when SR Ca load is high; and (3) by Ca diffusing from nearby CRUs that have just released Ca from the SR, also through CICR. The first 2 mechanisms are welldocumented experimentally, supporting the local control theory of Stern.¹⁸ According to local control theory, the third mechanism of spark-induced sparks is generally assumed to be unimportant during normal excitation-contraction cou-

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From the Department of Mathematics (R.R.), Loyola Marymount University, Los Angeles, Calif; Department of Physics (X.C.), Beijing Normal University, People's Republic of China; and Departments of Physiological Science (X.C., A.G.), Medicine (Cardiology) (A.G., J.N.W., Z.Q.), and Physiology (J.N.W.), David Geffen School of Medicine, University of California, Los Angeles.

Correspondence to Zhilin Qu, PhD, Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA, A2-237 CHS, 650 Charles E. Young Dr South, Los Angeles, CA 90095. E-mail zqu@mednet.ucla.edu

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pling. However, many experimental studies^{17,19} documenting the transitions from Ca spark to Ca wave indicate that spark-induced sparks become significant at some point as SR Ca load progressively increases. The exact point at which this occurs is not well-defined, but experiments by Parker et al²⁰ and Brum et al²¹ have shown evidence of sparks triggering additional sparks, or sparks occurring sequentially, especially along the Z-line. Because both LCCs and RyRs open stochastically, Ca sparks tend to occur sparsely and randomly at low LCC open probability and/or under strong Ca buffering conditions,^{22,23} but more regularly at higher LCC open probability and weak Ca buffering, eg, for sparks evoked by action potentials in normal rabbit myocytes.²⁴ Finally, once a CRU releases Ca, it becomes refractory to further Ca release for a certain time period, ie, there is a refractory period. Thus Ca sparks exhibit restitution, such that their amplitude depends on the time interval from the previous Ca spark.^{19,25,26}

To incorporate these general features into our model, we represented LCCs and RyRs using random-walk Markov models. Using computer simulation of our spatially distributed Ca cycling model together with nonlinear dynamics analysis, we show how 3 generic CRU properties, including "randomness" (the stochastic nature of Ca sparks), "refractoriness," and "recruitment" (the ability of 1 spark to recruit its neighboring CRUs to spark, or "spark-induced sparks"), interact synergistically to result in Ca alternans. Our simulations and theory agree with the experimental observations by Diaz et al^{6,7} that irregular, asynchronous Ca release is required for Ca alternans, with local Ca waves triggered during the large-release beat. However, we also show that a steep fractional release-load relationship emerges naturally as a collective behavior of the CRU network and Ca alternans can even occur when SR Ca content is held constant. This agrees with the experimental observations by Picht et al12 and Hüser et al13 that alternans in diastolic SR Ca load is not required for Ca alternans.

Non-standard Abbreviations and Acronyms		
CICR	calcium-induced calcium release	
CRU	calcium release unit	
DS	dyadic space	
jSR	junctional sarcoplasmic reticulum	
LCC	L-type calcium channel	
NSR	network sarcoplasmic reticulum	
PCL	pacing cycle length	
RyR	ryanodine receptor	
SR	sarcoplasmic reticulum	

Methods

Spatially Distributed Ca Cycling Model

We developed a quasi-2D spatially distributed Ca cycling model (Figure 1), which includes a network SR (NSR) domain and a myoplasmic (Myo) domain coupled via SR Ca release and uptake. As illustrated in Figure 1A, this model comprises a CRU network coupled via Ca diffusion in each domain. Each CRU contains (Figure 1B): a junctional SR (jSR), which is diffusively connected to the NSR (J_{SR}), and a dyadic space (DS), which is diffusively connected to the myoplasm (J_{DS}) . Extracellular Ca (J_{LCC}) enters the dyadic space through voltage-gated LCCs (5 channels per CRU), which open stochastically and are simulated by a simple Markov model (Figure 1C). Ca is released from the jSR (J_{RvR}) through its associated cluster of RyRs (100 channels per CRU) to the DS. The RyRs also open stochastically, and are simulated using a Markov model by Stern et al27 (Figure 1D), in which activation and inactivation of RyRs are regulated by Ca in DS (with no regulation by SR luminal Ca). Ca is either extruded from the cell via the Na-Ca exchanger (J $_{\rm NCX}$), or taken back up into the NSR via the SERCA pump (J_{up}). Because at the resting potential the Na-Ca exchanger always extrudes Ca, to maintain a stable Ca equilibrium state, we also added a background Ca current (J_b) to bring Ca into the myoplasmic domain. A network of 100×100 CRUs was used in this study. The differential equations and parameter values used in this study are detailed in the Online Data Supplement at http://circres.ahajournals.org.

Computer Simulation

We discretized the local NSR and the myoplasm domains of each CRU into 5×5 grids. Thus, a 100 $\times100$ CRU network was dis-



Figure 1. The spatially distributed Ca cycling model. A, Schematic plots (side view and top view) of a coupled CRU network. B, Detailed illustration of a CRU. C, Simplified Markov model of the LCC. "O" is the open state, and "I" and "C" are the closed states. The LCC is activated by voltage and inactivated by voltage and Ca (as indicated by "Ca" in the graph) in the dyadic space. D, The Markov model of the RyR from Stern et al.²⁷ "O" is the open state; "I", "R", and "C" are the closed states. RyR is activated and inactivated by Ca (as indicated by "Ca" in the graph) in the dyadic space.



Figure 2. Steep fractional release-load relationship. A, SR Ca depletion vs SR Ca load measured in simulation (filled circles) and transformed using Equation 1 with <Ca_b>=430 μ mol/L (open circles). Inset shows the voltage clamp wave form (top) and the SR Ca trace (bottom) illustrating the measurement of depletion vs load. SR Ca load was altered by changing the background Ca current (J_b) in the model (see Methods).

B, Fractional Ca release vs SR Ca load. The fractional SR release was calculated from A using SR depletion divided by SR load. The **gray** line is the best fit with the Hill function: $0.49 \times [Ca_{SR}]^{17.5}$ /($667^{17.5}$ +[$Ca_{SR}]^{17.5}$]. **C**, Spark count vs SR Ca load in the corresponding simulations in **A**. In simulation, a spark is defined as occurring when the Ca concentration in the dyadic space surges above 10 μ mol/L.

cretized into 2 coupled 500×500 grids. The differential equations were numerically simulated using an operator splitting method by advancing first the diffusion step and then the remaining reaction steps using a first-order forward Euler method with time step $\Delta t=5 \ \mu$ s. The stochastic transitions of LCC and RyR were updated asynchronously using an explicit method with a variable time step ($\Delta t=5 \ \mu$ s to 1 ms) adapted to the local Ca concentrations. The details of the numeric and simulation methods are presented in the Online Data Supplement.

Results

Steep Relationship Between SR Ca Release and SR Ca Load

A steep relationship between SR Ca release and SR Ca load (steep fractional release–load relationship), which plays an important role in regulating contractile force during normal excitation–contraction coupling, has been implicated in the genesis of Ca alternans, as described earlier. To explore the mechanism, we paced our CRU network model with a voltage clamp waveform to evoke Ca release from the SR. After achieving steady state, we recorded the whole-cell SR Ca content just before stimulation, and Ca depletion after stimulation (see inset of Figure 2A). By changing the amplitude of the background Ca current to alter the SR load, we obtained the relationship between SR Ca depletion and SR Ca load (Figure 2A, black symbols). The fractional release (Figure 2B) was

small and insensitive to SR Ca content when SR load was low but increased steeply between 600 μ mol/L and 700 μ mol/L, saturating at around 50%. For each SR load, we also calculated the total number of sparks (Figure 2C). The number of sparks increased slowly at low SR load, but increased steeply between 600 μ mol/L and 700 μ mol/L, beyond which the spark number saturated (at 10 000, the total number of CRUs in the system). To examine how fractional release is linked to sparks, we calculated the average whole-cell SR depletion $<\Delta$ Ca> by summating the Ca depletion attributable to each individual spark, ie:

(1)
$$<\Delta Ca > = \sum_{i=1}^{N_{spark}} (Ca_{SR} - Ca_b^i) / N_{total}$$
$$= (Ca_{SR} - \langle Ca_b \rangle) N_{spark} / N_{total},$$

where Ca_{SR} is the luminal SR Ca content before each release, Ca_b^i is the minimum SR Ca content of the ith CRU after the depletion (with $\langle Ca_b \rangle = \sum_{i=1}^{N_{spark}} Ca_b^i / N_{spark}$ being the average postdepletion content), N_{spark} is the number of sparks evoked, and N_{total} is the total number of CRUs. Using the spark data in Figure 2C and $\langle Ca_b \rangle = 430 \ \mu \text{mol/L}$, the predicted Ca depletion (open circles in Figure 2A) using Equation 1 agrees well with the direct measurements. Note that $\langle Ca_b \rangle = 430 \ \mu \text{mol/L}$ is close to the depletion levels seen in individual sparks (see Figure 3 and 4,



Figure 3. Ca transient in the Ca cycling model at slow pacing. A, Clamped voltage (top), whole-cell SR Ca concentration (black), and whole-cell myoplasm Ca concentration (red) vs time for PCL=1.4 second. B, Ca traces from jSR (black dashed) and dyadic space (red) at different spatial locations for the same simulation shown in A. The blue line in each graph shows the Ca fluxes through the LCCs in the corresponding CRUs. C, Snapshots of myoplasmic Ca spatial distribution taken at the moment of the Ca transient peaks for 3 consecutive beats. D, Ca snapshots from a small region (dashed box in beat #1 in C) show wave-like spread of Ca release.



Figure 4. Ca alternans in the Ca cycling model. A, Clamped voltage (top), whole-cell SR Ca concentration (black), and whole-cell myoplasm Ca concentration (red) vs time for PCL=500 ms. B. Ca traces from iSR (black dashed) and dyadic space (red) at different spatial locations for the same simulation shown in A. The blue line in each graph shows the Ca fluxes through the LCCs in the corresponding CRUs. C, Snapshots of myoplasmic Ca spatial distribution taken at the moment of the Ca transient peaks for the 6 consecutive beats shown in A. D, Ca snapshots from a small region (dashed box in beat #2 in C) show wave-like spread of Ca release.

and Online Figure IV). Therefore, in our model in which Ca release by individual CRUs is not regulated by SR luminal Ca, a steep fractional release-load relationship nevertheless emerges as a collective behavior of the network, in proportion to the fraction of CRUs in the network generating sparks. In other words, a steep release-load relationship is not required as a property of individual CRUs in order for the CRU network, as a whole, to exhibit this behavior. The behavior arises because when SR and myoplasm reach equilibrium, higher SR Ca content translates to a higher mean Ca concentration in myoplasm and DS. At higher resting Ca in DS, a smaller increment in Ca is needed to induce opening of RyRs. Therefore, as Ca increases, the probability of Ca sparks triggered either by Ca influx through LCC openings or by Ca released from the neighboring CRUs also increases in a nonlinear fashion, as shown in Figure 2.

From Random Ca Sparks to Whole-Cell Ca Alternans

When the CRU network model is paced periodically with a clamped voltage waveform at slow rates, the whole-cell Ca transient and diastolic SR Ca load are regular (Figure 3A). Although the summated Ca transient is regular, individual Ca sparks occur irregularly. In Figure 3B, we plot several traces of Ca from individual jSR spaces (Ca "blinks,"²⁸ black), dyadic spaces (Ca sparks, red), and LCC Ca fluxes (blue) in arbitrarily selected CRUs. It can be seen that Ca sparks, blinks, and LCC openings occur more or less randomly. A spark may occur with or without LCC openings, and LCC openings in a CRU may or may not cause a Ca spark. Sparks occurring without companion LCC openings are sometimes induced by neighboring sparks (spark-induced sparks). Blinks of small amplitude can occur in the absence of sparks at a given location, because Ca release in a CRU drains Ca from neighboring CRUs via diffusion in the NSR. We calculated the numbers of CRUs with LCC openings, total sparks, LCC-triggered sparks (sparks with LCC openings),

and recruited sparks (sparks without LCC openings), which are shown in Online Figure I (A). In this case, \approx 70% of the CRUs release Ca on each beat, of which 70% are LCC-triggered sparks, and 30% are spark-induced sparks. Because Ca sparks occur randomly, the spatial distribution of myoplasmic Ca exhibits a random spatial pattern, which changes from beat to beat (Figure 3C), similar to the irregular pattern observed during action potential clamps in mouse ventricular myocytes,²² although different from rabbit ventricular myocytes (see Discussion).²⁴ In addition, CRUs tend to release Ca in clusters because of spark-induced sparks, resulting in spreading Ca waves (Figure 3D), which are sporadic and abort locally. Space-time plots (line-scan) exhibit random and patch-like patterns, resembling the experimental data by Diaz et al⁶ (see Online Figure II).

As the pacing cycle length (PCL) decreases, whole-cell Ca alternans emerges. During alternans, both whole-cell myoplasmic Ca and SR Ca transients alternate in size from beat to beat (Figure 4A). The individual Ca sparks do not alternate but still occur randomly (Figure 4B), similar to the case of regular release (Figure 3B). However, the whole-cell spatial Ca pattern alternates from beat to beat (Figure 4C, see also the Online Video). Note that Ca sparks are sporadic and infrequent during the small Ca transients. During the large Ca transients, the sparks are still randomly distributed in space, but become more frequent. During alternans, the local waves occur during the large Ca transient (Figure 4D) but rarely during the small Ca transient, in agreement with the observations of Diaz et al.7 Also, notice that the number of CRUs triggered by LCC openings only alternates slightly (Online Figure I, B), indicating that many LCC openings during the small Ca beat do not successfully trigger Ca sparks. The recruitment rate (defined as the ratio of recruited sparks to LCC triggered sparks) alternates between 15% and 30% (Online Figure I, C). In a time-space plot (line scan), the alternating pattern (Online Figure III) resembles the experimental data from Diaz et al.7



Figure 5. Ca alternans does not rely on SR Ca load. A, Clamped SR Ca concentration (gray dashed) and myoplasmic Ca concentration vs time (PCL=500 ms). B, Ca depletion from SR vs SR Ca content as PCL increased gradually from 500 to 2000 ms in increments of 50 ms, with the initial 20 beats for PCL=500 ms ("stable alternans") and the last 10 beats for PCL=2000 ms ("regular depletion").

During both regular pacing and rapid pacing-induced alternans, the duration of Ca sparks varies from \approx 30 ms to >100 ms (Online Figure IV). The local SR Ca depletions (blinks) largely coincide with local Ca sparks, but the recovery of the local SR Ca content always exhibits a slow phase, reflecting the overall recovery of the global SR Ca content. In addition, the local SR Ca may deplete before (Online Figure IV, D) or after (Online Figure IV, E) the corresponding CRU spark. These results generally agree with the experimental observations by Zima et al,²⁹ and are attributed to the diffusion of Ca within the SR network.

Ca Alternans and the Steep Fractional Release–Load Relationship

To test whether a steep fractional release–load relationship is required for Ca alternans, as proposed in previous studies,^{4,14} we clamped the SR Ca to a constant value, and paced the system as in Figure 4. Ca alternans still occurred (Figure 5A), indicating that a steep release–load relationship is not required. However, we could only induce Ca alternans when Ca was clamped at a high level corresponding to the steep region of the fractional SR Ca release curve (between 600 and 700 μ mol/L), and over a limited range of cycle lengths. If the SR Ca was clamped at a level either below or above this, alternans did not occur.

To further analyze how SR Ca release is related to SR load, we used a "ramped pacing" protocol similar to that by Picht et al.¹² We initially paced the model at the control PCL of 500 ms, at which Ca alternans was already present, and then gradually increased the PCL to 2000 ms, at which Ca alternans was absent. A plot of the SR depletion versus SR load (Figure 5B) resembles the data from the experiment by Picht et al¹² (Online Figure V). In particular, note that the diastolic SR Ca content during periodic beating in the absence of Ca alternans (see Figure 5B) is significantly lower than the SR diastolic Ca immediately preceding the smallrelease beat during stable alternans. However the amount of Ca release during the periodic beating is greater in the absence of Ca alternans, indicating that the SR Ca release does not completely rely on SR Ca content.

A Dynamical Mechanism of Ca Alternans

In a recent theoretical study,³⁰ we demonstrated a perioddoubling bifurcation in an array of coupled randomly excitable elements subjected to periodic forcing and hypothesized that the same mechanism might be applicable to Ca alternans in cardiac myocytes (although no direct comparison was made). The 3 critical properties of the array required for this behavior included 2 properties assigned to the individual excitable elements (random activation and a refractory period), and 1 cooperative property between the elements (recruitment). Here, we revisit the theory in terms of Ca sparks and Ca alternans and directly compare the general theory to the modeling results.

We assume that α is the probability of a primary Ca spark (Figure 6A), activated either spontaneously (because of high SR load or leakiness) or by opening of LCCs in the CRU; γ is the probability of a primary Ca spark recruiting a neighboring CRU to spark (secondary sparks); and β the probability that a CRU activated on the previous beat remains refractory during the present beat. If, on the kth beat, N_k out of N_0 total CRUs were activated, then on the $(k+1)^{th}$ beat, the number of CRUs available to be activated are $A_k = N_0 - \beta N_k$, because βN_k CRUs are still in the refractory state. The number of primary sparks at the $(k+1)^{th}$ beat is then αA_k . The number of secondary sparks will be a fraction (*f*) of the remaining available (recovered) CRUs, ie, $(1-\alpha)A_kf$. Therefore, the total number of activated CRUs during the $(k+1)^{th}$ beat is:

(2)
$$N_{k+1} = \alpha A_k + (1-\alpha)A_k f = (N_0 - \beta N_k)[\alpha + (1-\alpha)f]$$

Equation 2 relates the number of Ca sparks in the present beat to the number of Ca sparks in the previous beat. What determines the fraction f? For an intuitive understanding, we show 2 schematic plots in Figure 6B for 2 beats in which CRUs have either mostly regained excitability (high recovery), or mostly remained refractory (low recovery). Because the spatial distribution of excitable and refractory CRUs is random in both cases, excitable CRUs are more isolated from each other in the case when most CRUs are refractory. This makes recruitment more difficult and leaves more potentially excitable CRUs unrecruited when the system is less recovered, and thus the recruitment rate is lower. Besides recovery, f also depends on primary spark rate α , recruitment probability γ , and the number of nearest neighbors. Using a meanfield approximation,³⁰ we explicitly derived f as (see the Online Data Supplement):

(3)
$$f(\alpha, \beta, \gamma, N_k) = 1 - [1 - \alpha \gamma (1 - \beta N_k / N_0)]^n$$

where *n* is the number of neighbors of a CRU. The recruitment rate, defined as the ratio of recruited sparks to primary sparks, ie, $(1-\alpha)A_kf'\alpha A_k$, is then:

recruitment rate =
$$(1 - \alpha)f/\alpha$$

(4)



Figure 6. Mean-field theory of Ca alternans. A, Illustration of different CRU states and spark-induced sparks. α is the intrinsic probability an individual CRU will spark, whereas γ is the probability that 1 sparking CRU will recruit another to spark. B, Schematic plots illustrating recruitment rate at a low and a high recovery beat. C, Recruitment rate (defined as the ratio of recruited sparks to primary sparks) vs recovered ratio (defined as the ratio of available CRUs to total CRUs) for different γ when $\alpha = 2/3$ (left) and for different α when γ =0.75. Lines are theoretical results of Equation 4, which are confirmed by numeric simulations (symbols). Numeric simulations were performed in a 100 \times 100 CRU array, in which we first randomly assigned recovered and uncovered CRUs with a certain ratio, and then applied the rules outlined in A to determine the primary and recruited sparks. D, Phase diagram of α - γ parameter space for β =0.98 and different number (n) of neighbors, obtained analytically using Eqs.2 and 3 (see the Online Data Supplement). E, Bifurcation diagram showing spark number vs parameter α by iterating Equation 2 with $\gamma = 0.75$, $\beta = 0.98$, and n=4. For each α , the first 100 transient

beats were dropped and the spark numbers in last 10 beats were plotted. **F** and **G**, Number of sparks vs the beat number from the nonalternating (α =0.5, γ =0.25, and n=4) and the alternating (α =0.8, γ =0.75, and n=4) regions in **D**.

The recruitment rate (Equation 4) for different α and γ is shown in Figure 6C along with the direct numeric results. Recruitment rate increases as γ increases, or as the number of recovered CRUs increases but decreases as α increases.

Inserting Equation 3 into Equation 2, the steady state equilibrium of the system and its stability can be explicitly analyzed (see the Online Data Supplement). The stability of the steady state depends on α , β , and γ , and also on the function for f (and its slope). When the steady state becomes unstable, alternans occurs. Behaviors of Equation 2 are illustrated in the α - γ parameter space in Figure 6D, with alternans occurring in the region above the line for each n (number of neighbors). In general, alternans occurs when α is intermediate (Figure 6E), γ is large, and β is large. Note that the large slope of the recruitment ratio curves shown in Figure 6C also occurs at intermediate α and large γ . Outside of the alternans regime, a transient alternans attributable to the initial condition can be observed but quickly converges to the stable steady state (Figure 6F). However, alternans is persistent in the alternans regime (Figure 6G) where the steady state is unstable.

The parameters α , β , and γ in Equation 2 do not explicitly appear in the Ca cycling model, but can be linked to physiological parameters in the physiologically detailed Ca cycling model to gain mechanistic insights into Ca alternans, as illustrated by the examples below:

1. The parameter α is affected by factors such as the open probabilities of the LCC and RyR channels and the Ca content of the myoplasm and the SR. For example, decreasing LCC open probability is analogous to decreasing α . If the LCC open probability is high (thus α is high), based on the theoretical predictions shown in Figure 6D and 6E, then inhibiting the LCC current (thereby lowering α) should promote alternans, which indeed occurs in our spatially distributed Ca cycling model (Figure 7A). This was also observed experimentally in voltage-clamped myocytes by partially blocking LCC.^{31,32} α can also be affected by extracellular Ca concentration [Ca]_o. Lower [Ca]_o corresponds to smaller α . Figure 7B shows that alternans occurs in the intermediate range of [Ca], in our Ca cycling network model, agreeing with the theoretical prediction that alternans occurs in the intermediate range of α . This also agrees with the experimental observations that overloading the cell with extracellular Ca can either promote³³ or suppress³⁴ Ca alternans.

2. The probability β that a CRU remains refractory after a previous spark can be attributed to either intrinsic RyR channel properties or RyR regulation by SR luminal Ca, such as by calsequestrin binding to the RyR protein complex.35,36 Our theory predicts that alternans can occur only when β is very large, indicating that either prolonging the refractory period or shortening the stimulation period (which increases β) will promote alternans if α and γ are also properly chosen. For example, Schmidt et al³⁵ showed in mouse heart that overexpression of calsequestrin promoted pulsus alternans and Restrepo et al37 showed in a modeling study that increasing calsequestrin concentration prolonged RyR refractoriness and promoted alternans, in agreement with our theoretical predictions. Figure 7C shows that, in our simulations using the spatially distributed Ca cycling model, alternans is promoted by decreased PCL,



Figure 7. Predictions of the CRU network model of Ca cycling. A, Peak myoplasmic Ca concentration vs the maximum LCC conductance (g_{Ca}) . B, Peak myoplasmic Ca concentration vs $[Ca]_o$. C, Peak myoplasmic Ca concentration vs PCL. D, Peak myoplasmic Ca vs CRU distance. In each graph, 5 to 10 peak myoplasmic Ca concentrations were plotted against each selected *x*-axis value.

consistent with the standard experimental method of inducing alternans by decreasing PCL.

3. The spatial cooperativity parameter γ , reflecting mainly the sensitivity of CICR, is affected by Ca level in the myoplasm, Ca diffusion rate, and the spacing between CRUs. For example, increasing CRU spacing makes recruitment less efficient (thus decreasing γ), which, based on our theory, suppresses alternans. This is demonstrated in the simulation results shown in Figure 7D. In their modeling study, Restrepo et al³⁷ showed that increasing the Ca diffusion rate (and thus increasing γ) promoted alternans, agreeing with our mean-field theory predictions. In addition, loading the cell with more Ca increases the sensitivity of RyRs to CICR, which enhances both the primary spark rate α and recruitment rate γ , thus promotes Ca alternans as [Ca]_o increases from low to high (Figure 7B).

Discussion

In this study, we developed a spatially distributed Ca cycling model to investigate how Ca sparks interact cooperatively to generate the steep fractional release-load relationship and Ca alternans. We show that Ca alternans emerges naturally as a result of 3 generic properties of the CRUs: randomness, refractoriness, and recruitment. Two of these 3 properties represent intrinsic CRU properties (random activation of Ca sparks and the refractory period of a CRU), whereas the third (recruitment, or spark-induced sparks) is governed by the spatial cooperativity between CRUs. In this Ca cycling model, Ca alternans does not causally rely on either regulation of RyR by SR luminal Ca, or a steep fractional release-load relationship, as proposed previously.⁴ This is not to imply that the latter regulatory features do not exist or are unimportant, but merely to show that they are not formally required to produce Ca alternans when a generic mechanism of RyR refractoriness, coupled with randomness and recruitment, is present.

Randomness

A typical myocyte consists of 10 000 to 20 000 CRUs with each CRU containing 5 to 20 LCCs and 50 to 300 RyRs.^{1,17,38–40} Because both LCC and RyR open randomly, Ca sparks are also naturally random, but their degree of randomness depends on the open probability of LCC and RyR. For example, if one assumes that only 1 LCC is needed to trigger a spark, and if a CRU has 10 LCCs, each with a low open probability of 0.05, the probability that none of the LCCs will open on a given depolarization is $(1-0.05)^{10}=0.6$, so that the probability of a spark is only 0.4. However, for the same CRU in which the open probability of LCC is 0.5, the probability that none of the LCC will open is reduced to $(1-0.5)^{10}=0.001$, so that a Ca spark will occur on virtually every beat. Equivalently, if the LCC open probability is high, but the probability of an LCC opening activating the RyRs is low, the Ca spark probability will also decrease. In our mean-field theory, α is the parameter describing the random excitability of the LCC and RyR channels. In the extreme case, when $\alpha = 1$, all CRUs will fire as primary sparks at each beat, no recruitment can occur and thus alternans cannot develop. As illustrated in Figure 6, randomness is also necessary for the steep nonlinear recruitment function f. If the recovered CRUs were not randomly distributed, it would be hard to image how such a nonlinear recruitment function f can emerge. In fact, alternans in the experiments by Diaz and colleagues^{6,7,32} was induced by reducing either LCC open probability (with LCC blockers or mildly depolarized voltage clamp pulses), or coupling fidelity (with RyR blockers or acidosis to reduce RyR open probability). In addition, they observed in their confocal imaging studies that asynchronous Ca release was needed for alternans. Their studies provide direct experimental support for our theoretical argument that randomness is necessary for alternans. Our prediction that random spark activation plays a key role in alternans might seem incompatible with the observation that when rabbit ventricular myocytes are paced with an action potential clamp at rates too slow to induce alternans, sparks occur regularly from the same sites.²⁴ However, we predict that when the rate is increased sufficiently to induce Ca alternans, this regular spark appearance will deteriorate and become asynchronous, because of the reduction in coupling fidelity as the rapid heart rate impinges on RyR refractoriness. This will be an important experimental test of the theory.

It has been shown experimentally that Ca release becomes asynchronous in diseased heart,^{41,42} which may be caused by a lower primary spark rate (α) resulting from remodeling processes, such as T-tubule disruption,^{43,44} altered excitation–contraction coupling, and altered cooperativity between RyRs within the same CRU. Lowering α enhances not only randomness, but also enhances recruitment (because more CRUs are available to be recruited), which may be one of the predisposing causes of alternans in ischemia,⁴⁵ heart failure,⁴⁶ and catecholaminergic polymorphic ventricular tachycardia.⁴⁷

Refractoriness

After RyRs in a CRU open and release Ca, they inactivate and require time to recover excitability. RyRs are the poreforming proteins mediating SR Ca release, but coassemble with a variety of accessory proteins that regulate their open probability and sensitivity to myoplasmic free Ca. Although the precise molecular basis of CRU refractoriness remains controversial, several nonexclusive mechanisms have been proposed. (1) Intrinsic RyR refractory period, as in the model proposed by Stern et al (Figure 1D): in the simplest mechanism, RyRs undergo conformational changes between different states, similar to other ligand-gated channels, one of which is a refractory state. (2) Direct regulation of RyR open probability by SR luminal Ca: in this mechanism, RyRs are activated by increased myoplasmic free Ca, but their sensitivity is also coregulated by a Ca-binding site in the C terminus of RyR, which senses SR luminal free Ca.48 (3) Indirect regulation of RyR open probability by SR luminal Ca: in this mechanism, RyRs are activated by increased myoplasmic free Ca, but the sensitivity is coregulated by calsequestrin, through its interaction with accessory proteins such as triadin and junctin in the RyR protein complex.⁴⁹

In terms of providing the refractoriness required in our analysis, any one of these 3 molecular mechanisms of RyR refractoriness is sufficient to account for alternans, whether SR luminal Ca regulation is absent (mechanism 1) or present (mechanisms 2 and 3). Although refractoriness is necessary for alternans in our theory, it is not sufficient without the other 2 factors (randomness and recruitment). Its role in alternans can be understood as follows (see also Figure 6B and 6C). Immediately before the beat with a large Ca transient, most of the CRUs are recovered (because they did not spark on the prior beat with a small Ca transient). Thus, available CRUs are densely distributed, such that their probability of recruiting secondary sparks is high (large f in Equation 2). Immediately afterward, however, most CRUs will be refractory, because they were just activated. Thus, on the next beat following a large Ca transient, few CRUs are available and they will be sparsely distributed, and recruitment of secondary sparks is therefore low (small f in Equation 2).

In agreement with our theoretical argument, increasing RyR refractoriness by transgenic overexpression of calsequestrin was shown in both experiments³⁵ and simulations³⁷ to promote alternans. On the other hand, Ca alternans was also reported to be enhanced in a transgenic mouse model of catecholaminergic polymorphic ventricular tachycardia, in which RyR refractoriness was decreased.⁴⁷ It will be interesting to explore further whether specific combinations of randomness, refractoriness, and recruitment can concomitantly promote both Ca alternans and Ca waves.

Recruitment

Recruitment, ie, the efficiency of spark-induced sparks, is determined by spatial cooperativity between CRUs. It is mediated by Ca diffusion and is sensitive to a variety of factors, including the Ca concentrations in the myoplasm and SR, the SR Ca uptake and leak rates, Ca buffering, and the spacing between adjacent CRUs. The process of sparkinduced sparks, combined with randomness and refractoriness, is crucial for the nonlinearity (recruitment function f in Equation 2) required for alternans. For example, as shown in Online Figure I, during steady state (PCL=1400 ms), the recruited sparks accounted for 30% of the total sparks (corresponding to recruitment rate 40%). During alternans (PCL=500 ms), the recruitment rate was around 30% on the large beat, but dropped to 15% on the small beat. Sparkinduced sparks are also the basis of myoplasmic Ca waves attributable to CICR,19,50 and therefore naturally link Ca waves to Ca alternans, as observed in experiments.5-7 Our simulations show that reducing CRU spacing increases recruitment rate and thus promotes alternans. This may be one of the predisposing causes of alternans in heart failure, in which remodeling has been shown to decrease CRU spacing.51 Whether spark-induced sparks occur under normal conditions is unclear, but they are promoted by conditions of Ca overload (increased extracellular Ca or fast pacing), increased RyR sensitivity to SR or myoplasmic Ca, decreased CRU distance, etc. These conditions are known to be present in disease states,51,52 consistent with the observation that Ca alternans and waves are promoted by heart failure and ischemia.

Steep Nonlinear Fractional Ca Release–Load Relationship

A steep nonlinear release–load relationship is a fundamental property of cardiac excitation–contraction coupling and plays an important role in the physiological regulation of the whole-cell Ca transient amplitude, and hence contractile force, in response to changes in heart rate and autonomic tone that alter the SR Ca load. Both myoplasmic and luminal SR Ca regulation of RyRs have been suggested as possible sources for the steep nonlinear relationship.⁵³ Here, we show that SR luminal Ca regulation of RyR is not strictly necessary for the steep release–load relationship and Ca alternans. The

underlying source of the steep release-load relationship is a dual increase in both SR Ca and myoplasmic Ca level. The increase of myoplasmic Ca increases the open probability of the RyR and thus increases the Ca spark probability of a CRU. In addition, as myoplasmic Ca increases, the probability of spark-induced sparks also increases. These 2 factors cause the number of sparks to increase steeply and nonlinearly as the SR load increases, resulting in a steep nonlinear release-load relationship. Although the occurrence of Ca alternans is always accompanied by a steep fractional release-load relationship as observed in experimental studies,4,6,7,11 a causal relation between the steep fractional release-load relationship and Ca alternans may not exist. In other words, both the steep fractional release-load relationship and alternans may be the result of enhanced CICR and spark recruitment, instead of one causing the other. On the other hand, although our model does not include SR luminal Ca regulation of RyR, we do not mean to imply that this feature is not important, only that in a minimal CRU network, it is not an absolute requirement for either a steep releaseload relationship or Ca alternans. In fact, experimental35,36,49,54 and modeling37,52 studies directly support luminal SR Ca regulation of RyR as playing important roles in Ca release and Ca alternans. However, it is more difficult to explain the dissociation of SR Ca load and release during Ca alternans as observed by Picht et al,¹² if release is tightly regulated by luminal SR Ca load under all conditions. It is intriguing to speculate that luminal SR Ca regulations may have evolved as a refinement to an already present property of the CRU network, to provide robustness and/or a greater dynamic performance range.

Limitations

In our study, we have focused on the minimum properties of a CRU network required to produce Ca alternans. Several limitations need to be addressed in future studies. (1) A real myocyte is a 3D system that contains a complex and heterogeneous CRU network coupled with a complex T-tubule network. (2) Only 1 of the 3 molecular mechanisms of refractoriness was studied in our Ca cycling model. (3) Many molecular signaling pathways regulate LCCs, RyRs, and SERCA pump. These molecular and subcellular factors undoubtedly play important roles in the genesis of Ca alternans. In addition, how intracellular Ca couples with membrane voltage to result in repolarization alternans and how the Ca alternans maintains synchronized in multicell tissue need to be investigated in future studies. Despite these limitations, our model and theory can explain the key experimental observations and provide a unifying general theory linking the steep fractional release-load relationship, Ca alternans, and Ca waves in cardiac myocytes.

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Disclosures

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Novelty and Significance

What Is Known?

- Pulsus alternans and T-wave alternans are associated with cardiac arrhythmias and sudden death, and calcium alternans has been assumed to be one type of cellular alternans responsible for T-wave alternans and pulsus alternans of the heart.
- Calcium alternans tends to occur under conditions of calcium overload and fast heart rates in normal cells and is exacerbated under diseased conditions such as heart failure and ischemia.

What New Information Does This Article Contribute?

- A computer model that simulates spatially distributed Ca sparks and whole-cell Ca alternans.
- A novel mechanism of Ca alternans that links whole-cell Ca alternans to the properties of Ca sparks.

The mechanisms of calcium alternans are not well understood and remain controversial. Using computer simulation and theoretical analysis, we developed a novel theory of calcium alternans that mechanistically links random, locally interacting calcium sparks to a stable whole-cell calcium alternans. We show that Ca alternans is a collective behavior of calcium sparks, driven by the synergistic interactions of 3 key properties of the calcium release units (CRUs): refractoriness of the CRUs, random activation of CRUs, and sparks inducing neighboring CRUs to spark. Through these 3 properties, one can link various molecular factors, such as calsequestrin regulation of the ryanodine receptors, and structural factors, such as the inter-CRU distance, to whole-cell alternans, providing a unified mechanistic understanding of calcium alternans attributable to seemingly unrelated causes.

Supplemental Materials

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I. Mathematical equations of the model

Notation and Conventions

Throughout this supplement we use single-letter subscripts to denote the four principal regions of the cell: myoplasm (m), network SR (s), junctional SR (j), and dyadic space (d). We suppress use of the spatial variable x except where explicitly needed. In cases where we refer to indexed positions for the CRUs, we use the superscript (i), with the additional understanding that $x^{(i)}$ is the location of the *i*th CRU.

Currents related to the myoplasm and SR domains are generally computed as "current densities" in units of concentration per time ($\mu M/ms$), with myoplasm as the reference volume, and are denoted with a capital *J*. The ratio of the myoplasm to SR volume is given as κ . Currents related to the local dyadic space and junctional SR compartments of a CRU are generally computed as "ionic fluxes" in units of ions per time, and are denoted with a capital *I*. At locations along the myoplasm or SR domains which come into contact with a CRU, these two types of currents are summed by first converting ionic fluxes to current densities (with units of concentration per time) by assuming the ions flow into or out of a local volume element ξ_m (or ξ_s) in the myoplasm (or SR), near the point of contact ¹. For consistency, we must have the restriction $\xi_m/\xi_s = \kappa$. See Section II for futher discussion.

Fundamental Equations

The time evolution of the concentration of calcium in the two-dimensional myoplasm and the network SR domain is modelled using a reaction-diffusion equation following the general form as by Dawson et al ¹. Spatially-continuous reaction terms couple the two domains and extrude calcium from the myoplasm domain. At discrete locations, additional reaction terms also couple the two domains via the CRUs, each of which contains a junctional SR compartment and a dyadic space compartment.

The system of equations for the evolution of calcium in the cell is

$$\beta_m(c_m)\frac{\partial c_m}{\partial t} = D_m \nabla^2 c_m + J_m$$
$$\frac{\partial c_s}{\partial t} = D_s \nabla^2 c_s + J_s$$
$$\beta_d(c_d)\frac{dc_d^{(i)}}{dt} = J_d^{(i)}$$
$$\frac{dc_j^{(i)}}{dt} = J_j^{(i)}$$

where $c_m(x)$ and $c_s(x)$ are the local concentrations in the myoplasm and network SR, respectively, and $c_d^{(i)}$ and $c_j^{(i)}$ are the calcium concentration in the *i*th dyadic space and *i*th junctional SR, respectively. The myoplasm and SR domains have diffusion coefficients D_m and D_s , respectively.

We assume calcium is buffered in the myoplasm (β_m) and dyadic spaces (β_d) only, by both dissolved calmodulin protein and SR-bound proteins, and that such buffering occurs rapidly enough to be modelled ² with the instantaneous capacity functions

$$\beta_m(c) = 1 + \frac{B_{sr}K_{sr}}{(c+K_{sr})^2} + \frac{B_{cd}K_{cd}}{(c+K_{cd})^2}$$
$$\beta_d(c) = 1 + \frac{B'_{sr}K'_{sr}}{(c+K'_{sr})^2} + \frac{B'_{cd}K'_{cd}}{(c+K'_{cd})^2}$$

The terms J_m , J_s , $J_d^{(i)}$ and $J_j^{(i)}$ represent the net current for each principal region, and are specified below.

Remaining physiological parameters for this section are defined in Online Table I.

Myoplasm flux equations

Ca enters and leaves the myoplasm due to uptake, exchange, and background leak currents, with net current density

$$J_m = -J_{up} - J_{NCX} + J_{bg} + J_{SRleak} + \xi_m^{-1} \sum_i \delta(x - x^{(i)}) I_{ds}^{(i)}$$

The proteins mediating the SR uptake, NaCa exchange, and cell background and SR leak currents are generally found distributed evenly throughout their respective regions, thus we model the corresponding current densities as spatially-continuous functions of position.

The SERCA uptake pump is modelled to be driven solely by the myoplasm Ca with the simple hill function

$$J_{up} = v_{up} \frac{c_m^2}{c_m^2 + k_{up}^2}$$

with a maximum velocity v_{up} and half-maximal concentration k_{up} .

The Ca current through the sodium-calcium exchange (NCX) pump, known to be a reversible current sensitive to both myoplasm Ca and membrane voltage, is modelled following the physiological model of Weber et al ³:

$$J_{NCX} = v_2 \frac{\left(e^{\eta\phi} [Na]_i^3 [Ca]_o - e^{(\eta-1)\phi} [Na]_o^3 c_m\right) \left(1 + k_{sat} e^{(\eta-1)\phi}\right)^{-1}}{\left(1 + \left(\frac{K_{mCa_{act}}}{c_m}\right)^3\right) \left(\begin{array}{c}K_{mCa_o} [Na]_i^3 + K_{mNa_o}^3 c_m + K_{mNa_i}^3 [Ca]_o \left(1 + \frac{c_m}{K_{mCa_i}}\right) + \right)}{K_{mCa_i} [Na]_o^3 \left(1 + \frac{[Na]_i^3}{K_{mNa_i}^3}\right) + [Na]_i^3 [Ca]_o + [Na]_o^3 c_m}\right)}$$

where $\phi = vF/RT$, and v is the net membrane potential (taking the outside of the cell as the reference value). The equation accounts for Ca and Na both inside and outside of the cell. Since our model does not describe Na dynamics as part of the action potential of the myocyte, we assign the internal Na ($[Na]_i$) a constant average value consistent with experimental data.

A background Ca leak current J_{bg} into and out of the cell is modelled as a linear function of membrane potential:

$$J_{bg} = -g_{bg}(v - 10)$$

A passive Ca leak current J_{SRleak} out of the SR into the cell is modelled as a linear function of the Ca gradient:

$$J_{SRleak} = g_{SRleak}(c_s - c_m)$$

The ionic flux $I_{ds}^{(i)}$ between the local myoplasm and the *i*th dyadic space, defined below, is a spatially-discrete current, present only at position $x^{(i)}$. The ionic flux, in units of ions per time, is normalized by the local myoplasm volume element ξ_m , as explained earlier, to produce a term in units of concentration per time ($\mu M/ms$).

Remaining physiological parameters for this section are defined in Online Table II.

SR flux equations

Ca enters and leaves the network SR due to uptake and transfer currents, with net value

$$J_s = \kappa (J_{up} - J_{SRleak}) - \xi_s^{-1} \sum_i \delta(x - x^{(i)}) I_{jsr}^{(i)}$$

The magnitude of the SR uptake and leak currents is rescaled by κ to account for the differing volumes of the SR and the myoplasm. The ionic flux of transfer to the local jSR is converted to units of concentration per time by ξ_s . The equation for the junctional SR transfer flux (I_{jsr}) is below.

Junctional SR equations

The net current density of Ca for the *i*th junctional SR (with volume V_i) is

$$J_{j}^{(i)} = \left(I_{jsr}^{(i)} - I_{ryr}^{(i)}\right)V_{j}^{-1}$$

in which $I_{ryr}^{(i)}$ is as described below, and

$$I_{jsr}^{(i)} = g_{jsr} \left(c_s(x^{(i)}) - c_j^{(i)} \right)$$

gives a first-order refilling of Ca from the network SR to the jSR with refilling rate rate g_{sr} .

Remaining physiological parameters for this section are defined in Online Table III.

Dyadic space equations

The net current density of Ca for the *i*th dyadic space, including that flowing through the stochastic RyR and LCC channels, is

$$J_d^{(i)} = \left(-I_{ds}^{(i)} + I_{lcc}^{(i)} + I_{ryr}^{(i)}\right) V_d^{-1}$$

The transfer flux I_{ds} from the dyadic space to the local myoplasm is dependent on the local gradient with flux rate g_{ds} :

$$I_{ds}^{(i)} = g_{ds} \left(c_d^{(i)} - c_m(x^{(i)}) \right)$$

Ca flux *into* the dyadic space, via either the L-type Ca channel (LCC) or the RyR channel, is the major route of entry of Ca into the cell as well as of central importance in this work. Because this entry is governed by a small number of interacting ion channels in each dyadic space, we represent the current through both types of channels as a random walk on simple Markov models, as described below.

Remaining physiological parameters for this section are defined in Online Table III.

Model for current through L-type calcium channels

In each dyadic space is a small cluster of L-type calcium channels; the kinetic of each channel are represented independently with a minimal 3-state Markov model, adapted from Hinch et al ⁴ (see Online Figure VI). The transition rates into and out of each state are computed locally for each CaRU, as they are dependent on the local dyadic Ca, and are given by

$$\alpha_1 = a_1 \frac{\theta + 0.0625}{14\theta + 0.0625}$$
$$\beta_1 = b_1 c_d^{(i)} \frac{\theta + 0.0625}{\theta + 1.0}$$
$$\alpha_2 = a_2 \frac{\theta}{\theta + 1}$$
$$\beta_2 = b_2$$

where $\theta = \exp((v+2)/7)$, v is the membrane voltage of the cell, and a_1 , a_2 , b_1 , and b_2 are adjustable constants.

There are n_L LCC channels in a single dyadic space, and each channel is simulated independently. Let $L^{(i)} \in \{0, 1, ..., n_L\}$ be a stochastic variable indicating the number of LCC channels in the open (O) state at any given time in the *i*th dyadic space. Then the total ionic flux through all LCC channels of the *i*th dyadic space is given by the voltage-dependent Goldman-Hodgkin-Katz equation

$$I_{lcc}^{(i)} = L^{(i)}g_{Ca}P_{Ca}\frac{2\phi(\beta_{Ca}[Ca]_o - e^{2\phi}c_d^{(i)})}{(e^{2\phi} - 1)}$$

where, again, $\phi = vF/RT$.

Remaining physiological parameters for this section are defined in Online Table IV. The dynamics of the stochastic variable $L^{(i)}$ are described in the section "Numerical Methods".

Model for current through RyR channels

In each dyadic space is a larger cluster of RyR calcium channels; the kinetics of each channel are represented with a minimal 4-state Markov model, adapted from Stern et al ⁵, which described the channel as having closed (C), open (O), inactivated (I) and refractory (R) states (see Online Figure VI). The transition rates for the two "horizontal" transitions (C to O, and R to I) are equivalent, with forward rate $k_a^+ \left(c_d^{(i)} \right)^2$ dependent on the square of dyadic calcium, and constant reverse rate k_a^- . Similarly, the transition rates for the two "vertical" transitions (O to I, and C to R) are equivalent, with forward rate $k_b^+ c_d^{(i)}$ and reverse rate k_b^- .

There are n_R RyR channels in a single dyadic space, and each channel is simulated independently. Let $R^{(i)} \in \{0, 1, ..., n_R\}$ be a stochastic variable indicating the number of RyR channels in the open (O) state at any given time in the *i*th dyadic space. We then assume the total ionic flux flowing through the entire RyR cluster of the *i*th dyadic space is dependent on the gradient between the local jSR and dyadic space, and is given by

$$I_{ryr}^{(i)} = R^{(i)}g_{ryr}\left(c_{j}^{(i)} - c_{d}^{(i)}\right)$$

Remaining physiological parameters for this section are defined in Online Table V. The dynamics of the stochastic variable $R^{(i)}$ are described in the section "Numerical Methods".

Membrane voltage pacing

In the absence of a dynamical model of voltage, we adopt a simple repeating waveform of period PCL (pacing cycle length) in which the voltage rise from v_{rest} to an action potential plateau value v_{ap} . After 3/4 of the action potential duration (APD) has elapsed, the voltage returns linearly to v_{rest} . For $t' = t \mod PCL$ within each cycle,

$$v(t') = \begin{cases} v_{ap} & \text{for } 0 \le t' < \frac{3}{4}APD \\ v_{ap} + \frac{v_{rest} - v_{ap}}{\frac{1}{4}APD} \left(t' - \frac{3}{4}APD\right) & \text{for } \frac{3}{4}APD \le t' < APD \\ v_{rest} & \text{for } APD \le t' < PCL \end{cases}$$

Remaining physiological parameters for this section are defined in Online Table VI.

II. Numerical methods

Diffusion and flux

The governing equations are advanced through time using a first-order forward Euler method with time step $\Delta t = 0.005 \text{ ms}$ and mesh spacing $\Delta x = 0.2 \mu m$. * In each timestep, the two concentration state variables c_m and c_s are updated twice, following an operator-splitting scheme ⁶, applying first the reaction (flux) terms and then the diffusion terms. Thus at timestep *n* (denoted with brackets), values are first updated to an intermediate value (denoted by timestep *n'*) with the following scheme:

$$c_{m}[n'] = c_{m}[n] + \Delta t \beta_{m}(c_{m}[n])^{-1} J_{m}[n]$$

$$c_{s}[n'] = c_{s}[n] + \Delta t J_{s}$$

$$c_{d}^{(i)}[n'] = c_{d}^{(i)}[n] + \Delta t \beta_{d}(c_{d}^{(i)}[n])^{-1} J_{d}^{(i)}[n]$$

$$c_{j}^{(i)}[n'] = c_{j}^{(i)}[n] + \Delta t J_{j}^{(i)}[n]$$

The spatial diffusion step is then applied to c_m and c_s (using superscripts *i* and *j* to denote position $\langle i\Delta x, j\Delta x \rangle$ in the x-y plane):

$$c_m^{i,j}[n+1] = c_m^{i,j}[n'] + \frac{D_m \Delta t}{\Delta x^2} \beta_m (c_m^{i,j}[n'])^{-1} \left[c_m^{i-1,j}[n'] + c_m^{i+1,j}[n'] + c_m^{i,j-1}[n'] + c_m^{i,j+1}[n'] - 4c_m^{i,j}[n'] \right]$$

$$c_s^{i,j}[n+1] = c_s^{i,j}[n'] + \frac{D_s \Delta t}{\Delta x^2} \left[c_s^{i-1,j}[n'] + c_s^{i+1,j}[n'] + c_s^{i,j-1}[n'] + c_s^{i,j+1}[n'] - 4c_s^{i,j}[n'] \right]$$

for Ca in the myoplasm (c_m) and network SR (c_s) .

Ion channel kinetics

The remaining state variables in the model are the ion channel states for each release unit, represented as continuous-time discrete-state Markov models. These are updated stochastically, and asynchronously to the concentration variables, using a Monte Carlo scheme with an adaptive timestep. For the purposes of stochastic simulation, the LCC and RyR channel kinetics are formulated in the style of a chemical master equation. Because each dyadic space is treated as a single-pool element, with no information about its spatial arrangement (other than the local volume), we assume that in the *i*th CRU every channel of a given type "sees" the same environment and thus has identical transition rates. Thus we can treat each channel type as a group, tracking only the number of channels in each possible state ⁷. We note here that because of the nonconstant nature of the time-dependent transition rates, we resort to explicit first-order methods and do not consider more efficient methods such as "tau-leaping" ⁷.

^{*}Although diffusion in this model is not explicitly three-dimensional, we can conceptually employ a "depth" term Δz so that we may refer to volume in ordinary units (μm^3). Depths of $\Delta z_{myo} = 5 \ \mu m$ and $\Delta z_{nsr} = 0.2 \ \mu m$ are consistent with the local volume elements $\xi_m = 0.2 \ \mu m^3 = (\Delta x)(\Delta x)(\Delta z_{myo})$ and $\xi_s = 0.008 \ \mu m^3 = (\Delta x)(\Delta x)(\Delta z_{nsr})$, and also maintain the volume ratio $\kappa = 25 = \Delta z_{myo}/\Delta z_{nsr}$, as given in the previous section. These Δz terms do not appear in any equations in this supplement, and are invoked here only for purpose of explanation.

In what follows, we present a general method for numerical simulation of a cluster of ion channels of a given type. In Section I we explicitly referred to the *i*th CRU by using the (i) index notation; here we suppress this notation, with the understanding that the algorithm is independently executed for each CRU and for each of the two channel clusters (RyR or LCC) within that CRU.

For an arbitrary cluster, let there be N_c ion channels following a markov state model that has N_s states labelled $\{1, \dots, N_s\}$, and N_t possible transitions between states with transition rates λ_{jk} , with $1 \le j, k \le N_s$, $j \ne k$ for transition between state j and state k. Let s_j be the number of channels in the *j*th state (so that $\sum_j s_j = N_c$). We assume that in a sufficiently small time step, only one transition will take place such that for some j and $k, s_j \rightarrow s_j - 1$ and $s_k \rightarrow s_k + 1$ (that is, the count of one state increase while the count of the other decreases) and no other changes take place.

Because the local Ca concentration values (which determine the transition rates) are constantly changing, what qualifies as a "sufficiently small" timestep is also variable, and we wish to take advantage of those epochs when the transition rates are low by adapting the local computational timestep δt for that CRU. We first choose a *minimum* local time step ε such that it is both much smaller than, and evenly divisible into, Δt , the global timestep of the diffusion computation step above. After each update step, we will compute the next adaptive time step δt as a multiple of this minimum value (ie, $\delta t = n\varepsilon$ for $n \in \{1, \dots, \Delta t/\varepsilon\}$). The transition probabilities are computed using this local timestep, an update step is taken, and then a new local time step is computed.

To choose an appropriate value for δt , consider the sum $\sum_{j,k} s_j \lambda_{jk} = \lambda$ as a "total" transition rate. Invoking a core property of such Markov models, we assume that the probability that the waiting time t' until the next transition is larger than δt is $P(t' > \delta t) = e^{-\lambda \delta t}$. Because calling the exponential (or log) function is computationally expensive, we use the common approximation $P(t' > \delta t) \approx \lambda \delta t$, and aim to limit the error in this approximation by imposing the bound $P(t' > \delta t) \leq \tau$ for some acceptable tolerance level (we choose $\tau = 0.10$), which leads to the selection

$$\delta t = \frac{\tau}{\lambda}$$

or alternatively, $n = \frac{\tau}{\lambda \varepsilon}$, where *n* is the number of minimum time steps ε we wish to wait until again reevaluating the channel states.

Having adapted the local timestep to the current transition rates, we can then implement a simple Monte Carlo update to choose which of the transitions will occur (if any). We must first make two definitions. First, assign the values $\lambda_{(l)}$, for $l \in \{1, \dots, N_t\}$, as an arbitrary ordering of the set of transition probabilities $s_j \lambda_{jk} \delta t$, for $1 \le j, k \le N_s$, $j \ne k$. Second, let $S_k = \sum_{l=1}^k \lambda_{(l)}$ create a partition of the interval [0, 1] into $0 < S_1 < S_2 < \cdots < S_{N_t} < 1$. Then, the following numerical algorithm is used:

- 1. Compute the individual transition probabilities $\lambda_{(l)}$ as well as the sum $\sum_{j,k} s_j \lambda_{jk} = \lambda$
- 2. Compute the local timestep $\delta t = \min(\tau/\lambda\varepsilon, \Delta t)$; wait this time.
- 3. Draw a uniform random number $r \in [0, 1]$. Update the channel states by making the *i*th transition corresponding to $S_{i-1} \le r < S_i$. If $S_{N_t} < r < 1$ then no transition takes place.

III. Derivation of the iterated map model

Consider an array of CRUs, each of which is either excited (ie, in state "1") or resting (in state "0"). Assume that each CRU has *n* total nearest neighbors with which it can interact. To construct the probability $P_{k+1}[1]$ of a CRU being in state 1 on the next beat k + 1, we first need to define another quantity, the joint probability $P_k^a(m)$ that, on the previous beat *k*, the CRU and exactly *m* of its neighbors are *available* to spark, given their behavior on the previous beat *k* (they can be available either because they did not spark, or they did spark, and recovered from their refractory period early enough to spark again). Then we can write the following master equation:

$$P_{k+1}[1] = \sum_{m=0}^{n} P_k^a(m) \left[\alpha + (1-\alpha) \sum_{j=0}^{m} B_m(P_k^a\alpha; j) \omega(j) \right]$$

where the large term in brackets states that a CRU could spark either by primary excitation (with probability α) or, if not, then by neighbor recruitment, which itself depends on how many neighbors have also fired on the same beat. The inner sum accounts for *j* of *m* possible neighbors firing, where $B_m(P_k^a\alpha; j) = {m \choose j} (P_k^a\alpha)^j (1 - P_k^a\alpha)^{(m-j)}$ is the binomial probability that *j* out of *m* neighbors fire (each having independent probability $P_k^a\alpha$, where P_k^a is the probability that the CRU is available after beat *k*, *independent* of the recent behavior of its neighbors), and $\omega(j)$ is the probability that the central unit will be excited given *j* neighbors did fire. Because only the *number* of excited neighbors in the neighborhood, and not the *location* (ie, north, south, east, or west), matters, we need only count the total number of neighbors exicted.

The function $\omega(j)$ describes the receptiveness of a unit to recruitment by its surrounding neighbors in the lattice; it should increase monotonically with *j* and saturate to 1. A simple function that meets these criteria is $\omega(j) = 1 - e^{-sj}$ for some scaling parameter *s*. If we identify $\gamma = \omega(1) = 1 - e^{-s}$ as the probability that a *single* neighbor can recruit a unit, we can rewrite $\omega(j)$ as

$$\boldsymbol{\omega}(j) = 1 - (1 - \boldsymbol{\gamma})^j$$

where we have now characterized $\omega(j)$ as the probability that *at least one* recruitment, out of *j* total chances and each with independent chance γ , will take place.

Returning to the master equation, we now make the simplying assumption that neighboring CRUs are independent from beat to beat, in the sense that whether one CRU sparked on the previous beat is not correlated with whether any of its neighbors fired. This implies that the joint probability $P_k^a(m)$ that the CRU and exactly $m \le n$ of its neighbors are available to spark is equal to the product

$$P_k^a(m) = P_k^a B_n(P_k^a;m)$$

where P_k^a is defined as before. (We note that this assumption implies a randomized, "well-mixed system", such that any spatial inhomogeneities that might emerge over repeated application of the update rules are ignored.)

Making the appropriate substitutions, the master equation now becomes

$$P_{k+1}[1] = P_k^a \sum_{m=0}^n B_n(P_k^a;m) \left[\alpha + (1-\alpha) \sum_{j=0}^m B_m(\alpha;j)\omega(j) \right]$$
$$= P_k^a \sum_{m=0}^n B_n(P_k^a;m) \left[\alpha + (1-\alpha)(1-(1-\alpha\gamma)^m) \right]$$
$$= P_k^a \left[\alpha + (1-\alpha)(1-(1-P_k^a\alpha\gamma)^n) \right]$$

Finally, we make the mean-field assumption that in an array of N_0 CRUs, the number N_{k+1} that spark on the next beat is the expected value

$$N_{k+1} = N_0 P_{k+1}[1]$$

and that the number A_k of CRUs that are available to spark following beat k is

$$A_{k} = N_{0}P_{k}^{a}$$

= $N_{0} (P_{k}[0] + (1 - \beta)P_{k}[1])$
= $N_{0} - \beta N_{k}$

where β is the probability that a CRU is refractory following a spark. Making these final substitutions, we have the iterated map equation for the number of sparks:

$$N_{k+1} = A_k \left[\alpha + (1 - \alpha) (1 - (1 - \frac{A_k}{N_0} \alpha \gamma)^n) \right]$$

As given in the main text, we identify the probability of a secondary spark as $f = 1 - (1 - \frac{A_k}{N_0} \alpha \gamma)^n$ to yield

$$N_{k+1} = A_k \left[\alpha + (1 - \alpha) f \right]$$

IV. Linear stability analysis of the iterated map model

For convenience, we rewrite the iterated map model (Eqs. 2 and 3 from the main text):

$$N_{k+1} = (N_0 - \beta N_k) [\alpha + (1 - \alpha)f]$$
(I)

where *f* is

$$f(\alpha, \beta, \gamma, N_k) = 1 - [1 - \alpha \gamma (1 - \beta N_k / N_0)]^n$$
(II)

At steady state, the spark number N_s satisfies (since $N_{k+1}=N_k=N_s$):

$$N_{\rm s} = (N_0 - \beta N_{\rm s})[\alpha + (1 - \alpha)f]$$
(III)

Assume the system begins at the steady state and a small perturbation δN_k is added to the kth beat, i.e., $N_k = N_s + \delta N_k$, which results in a deviation δN_{k+1} to the (k+1)th beat, i.e., $N_{k+1} = N_s + \delta N_{k+1}$. Inserting N_k and N_{k+1} into Eq.I, one obtains:

 $N_{s} + \delta N_{k+1} = [N_{0} - \beta(N_{s} + \delta N_{k})][\alpha + (1 - \alpha)f(\alpha, \beta, \gamma, N_{s} + \delta N_{k})]$ (IV) Using a Taylor expansion and Eq.III, one has:

$$\delta N_{k+1} = -\beta [\alpha + (1-\alpha)f] \delta N_k + (N_0 - \beta N_s)(1-\alpha) \frac{df}{dN_k} \delta N_k$$
(V)

where

$$\frac{df}{dN_{K}} = \frac{df}{da_{k}}\frac{da_{k}}{dN_{k}} = -\frac{\beta}{N_{0}}\frac{df}{da_{k}}$$
(VI)

and $a_k = (1 - \beta N_k / N_0)$ is the proportion of available CRUs. Inserting Eq.VI into Eq.V, one has

$$\delta N_{k+1} = \{-\beta [\alpha + (1-\alpha)f] - \beta (1-\beta N_s/N_0)(1-\alpha)\frac{df}{da_k}\} \delta N_k = \lambda \delta N_k$$
(VII)

where

$$\lambda = -\beta [\alpha + (1 - \alpha)f + (1 - \beta N_s / N_0)(1 - \alpha)\frac{df}{da_k}]$$
(VIII)

After several (j) iterations, the perturbation will become:

$$\partial N_{k+j} = \lambda^j \partial N_k \tag{IX}$$

From Eq.IX, if $|\lambda|>1$, the perturbation grows, indicating that the steady state is unstable. In addition, if $\lambda<-1$, the perturbation alternates in positive and negative values, resulting in an alternating pattern of spark numbers. Therefore, the criterion for alternans to occur is $\lambda<-1$ with the critical case as $\lambda=-1$. Therefore, based on Eq.VIII, a larger β and steeper recruitment fraction f (whose slope is determined by α and γ , see Online Figure VII) are in favor of the instability leading to spark alternans (Online Figure VIII).

V. References

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VI. Supplemental Tables

Online Table I: Parameter	values for	diffusion a	and buffering equations	

Symbol	Definition	Value
D_m	Diffusion coefficient of Ca in myoplasm	$0.35 \ \mu m^2 m s^{-1}$
D_s	Diffusion coefficient of Ca in SR	$1.4 \ \mu m^2 m s^{-1}$
κ	Ratio of myoplasm to network SR volume	25.0
ξ_m	Local volume element for myoplasm	$0.200 \ \mu m^3$
ξ_s	Local volume element for network SR	$0.008 \ \mu m^3$
B_{sr}	Concentration of SR-bound buffer (myoplasm)	7.0 μM
K_{sr}	Dissociation constant of SR-bound buffer (myoplasm)	0.3 μM
B_{cd}	Concentration of calmodulin buffer (myoplasm)	15.0 μM
K_{cd}	Dissociation constant of calmodulin buffer (myoplasm)	13.0 μM
B'_{sr}	Concentration of SR-bound buffer (dyadic space)	47.0 μM
K'_{sr}	Dissociation constant of SR-bound buffer (dyadic space)	0.6 µM
B'_{cd}	Concentration of calmodulin buffer (dyadic space)	24.0 µM
K'_{cd}	Dissociation constant of calmodulin buffer (dyadic space)	7.0 μM

Online Table II: Parameter values for SERCA (uptake), NCX, and background leak currents

Symbol	Definition	Value
v _{up}	Maximum pump rate of SERCA	$0.32 \mu M m s^{-1}$
k_{up}	Half-maximal activation constant for SERCA	$1.0 \ \mu M$
v_2	Adjustable pump rate constant of NCX	$0.4 \ \mu M m s^{-1}$
η	NCX voltage sensitivity constant	0.35
F	Faraday's constant	96.5 $C mmol^{-1}$
R	Gas constant	$8.314 J M^{-1} K^{-1}$
Т	Temperature	310 K
$[Ca]_o$	External [Ca]	1.80 <i>mM</i> *
$[Na]_o$	External [Na]	136 <i>mM</i>
$[Na]_i$	Internal [Na]	10.0 <i>mM</i>
$K_{mCa_{act}}$	Allosteric Ca inactivation constant	0.11 μM
K_{mCa_o}	External Ca sensitivity constant	1.30 <i>mM</i>
K_{mNa_o}	External Na sensitivity constant	87.5 <i>mM</i>
K_{mCa_i}	Internal Ca sensitivity constant	3.59 μM
K_{mNa_i}	Internal Na sensitivity constant	12.3 <i>mM</i>
k _{sat}	NCX saturation constant	0.27
g_{bg}	Rate of background membrane leak	$4.0 \cdot 10^{-5} \mu M m s^{-1} m V^{-1}$
	Rate of SR leak	$4.0 \cdot 10^{-6} \mu M m s^{-1}$

* These values may have been modified as noted in text

Symbol	Definition	Value
8 jsr	Flux rate from network to junctional SR	$0.1 \mu m^3 m s^{-1}$
8ds	Flux rate from dyadic space to myoplasm	$0.303 \ \mu m^3 m s^{-1}$
V_d	Volume of a single dyadic space	$1.26 \cdot 10^{-3} \ \mu m^3$
V_{j}	Volume of a single junctional SR	$0.1 \ \mu m^3$

Online Table III: Parameter values for other CRU fluxes

Online Table	IV: Parameter	values for LCCs
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Symbol	Definition	Value
<i>g</i> Ca	Adjustable LCC current multiplier	1.0 *
P_{Ca}	Ca Permeability	$0.913 \ \mu m^3 s^{-1}$
β_{Ca}	Ca partition coefficient	0.341
a_1	LCC rate constant (I to C)	$3.23 \mu s^{-1}$
a_2	LCC rate constant (C to O)	$0.30 ms^{-1}$
b_1	LCC rate constant (C to I)	$0.154 \ \mu M^{-1} m s^{-1}$
b_2	LCC rate constant (O to C)	$1.0 \ ms^{-1}$
n_L	Number of L-type Ca channels per dyadic space	5
	1 1 1 1 1 1 1 1 1 1 1	

* These values may have been modified as noted in text

Online Table	V: Parameter	values for	RyR	channels
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Symbol	Definition	Value
k_a^+	Opening rate coefficient (C to O / R to I)	$0.005 \ \mu M^{-2} m s^{-1}$
k_a^-	Closing rate coefficient (O to C / I to R)	$1.0 \ ms^{-1}$
k_{h}^{+}	Inactivation rate coefficient (C to R / O to I)	$0.00075 \ \mu M^{-1} m s^{-1}$
k_{h}^{-}	Recovery rate coefficient (R to C / I to O)	$0.003 \ ms^{-1}$
gryr	Flux rate from jSR to dyadic space	$0.000205 \ \mu m^3 m s^{-1}$
n_R	Number of RyR channels per dyadic space	100

Online Table VI: Parameter values for action potential

Symbol	Definition	Value
vap	Peak voltage during action potential	10 mV
<i>v_{rest}</i>	Resting voltage during diastole	-80 mV
APD	Action potential duration	200 ms
PCL	Pacing cycle length	$200-1000~ms$ \star

 \star These values may have been modified as noted in text

VII. Supplemental figures



Figure I. A-C: Total sparks (green squares), primary sparks (blue triangles), recruited sparks (red circles), and number of CRUs accompanied by LCC openings (red diamonds), measured from the simulations (Fig.3 and Fig.4). **A**. Steady state (no alternans) at PCL=1400 ms. **B**. Alternans at PCL=500 ms. **C**. Recruitment ratio calculated as the number of recruited sparks over the total number of sparks for PCL=500 ms. **D-F**. Same as A-C but from the iterated map model (theory). **D**. Steady state (no alternans) for α =0.55, β =0.2, and γ =0.35. **E**. Alternans for α =0.75, β =0.98, and γ =0.75 . **F**. The recruitment ratio of E.

Although α , β , and γ cannot be directly obtained from the Ca cycling model, we can estimate their values using the simulation results and the theory, as described below:

For PCL=1400 ms (panel A), out of the total 10000 CRUs, there are on average 6640 total sparks, of which 4670 are primary (due to LCC openings), and 1970 are secondary (recruited). The primary sparks satisfy: number of primary sparks= $\alpha A_k = \alpha (N_0 - \beta N_k)$, where N_k is the total number of sparks and is the same for each beat. Inserting numbers for total CRUs, the primary sparks, and the total sparks into this equation, one has: $4670 = \alpha (10000 - 6640\beta)$. Using the secondary spark formulation, i.e., secondary sparks= $(1-\alpha)A_kf$, and the function f in Eq.3 with n=4, one has: $(1-\alpha)[1-(1-0.467\gamma)^4]/\alpha=0.197/0.467$. We cannot obtain the three unknowns from the two equations, but if we set $\beta=0$, for example, we have $\alpha=0.467$ and $\gamma=0.23$; if $\beta=0.2$, we

have $\alpha=0.54$, and $\gamma=0.34$. For PCL=500 ms (panel C), for the high beat, there are (on average) 8360 total sparks, 6411 primary ones, and 1949 secondary ones; for the low beat, there are 2237 total sparks, 1909 primary ones, and 328 secondary ones. The primary sparks for the high beat satisfy: $\alpha(10000-2237\beta)=6411$; and for the low beat satisfy: $\alpha(10000-8360\beta)=1909$. Using these two equations, we obtain $\alpha=0.8$, and $\beta=0.91$. Even using these numbers and the formulation of the secondary sparks, we cannot obtain a completely consistent γ . One of the reasons may be that in the simulation of the Ca cycling model, there is sequential recruitment (i.e., spark \rightarrow spark) in the same beat to facilitate Ca waves, but in our mean-field theory, we assumed only one step recruitment. Nevertheless, this method, although incomplete, can result in a good quantitative estimate of the parameters. The results from the iterated map shown in D-F were obtained using α , β , and γ that are similar to the estimated values, which resulted in similar results as those from the computer simulation (compare panels A-C with panels D-F).



Figure II. A. Line-scans of regular Ca release in experiments by Diaz et al. B. Line-scans from our simulation shown in Fig.3 during slow pacing.



Figure III. A. Line-scans during Ca alternans in experiments by Diaz et al. B. Line-scans from our simulation shown in Fig.4 during fast pacing.



Figure IV. A-E. Five examples of Ca sparks and blinks by recordings of Ca concentration from the jSR (dashed blue) and dyadic space (red) of five CRUs.



Figure V. A. SR depletion vs. load (left) and SR Ca content vs. time from a simulation using a "ramp pacing" protocol. **B**. The experimental recordings from Picht et al.



Figure VI. Markov models for LCCs (left) and RyR channels (right). The rate constants are detailed in the Online text.



Figure VII. Plots of Eq.II (n=4) for different γ (α =2/3) and α (γ =3/4).



Figure VIII. Spark alternans region (above the lines) for two different β , and n=4.