Dependency of Calcium Alternans on Ryanodine Rece

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Abstract

Background

Rapid pacing rates induce alternations in the cytosolic calcium concentration caused by fluctuations in calcium rele
However, the relationship between calcium alternans and refractoriness of the SR calcium release channel (RyR2)

Methodology/Principal Findings

To investigate how ryanodine receptor (RyR2) refractoriness modulates calcium handling on a beat-to-beat basis u
used a mathematical rabbit cardiomyocyte model to study the beat-to-beat calcium response as a function of RyR
were constructed depicting the beat-to-beat response. When alternans was observed, a novel numerical clamping
was caused by oscillations in SR calcium loading or by RyR2 refractoriness. Using this protocol, we identified regi
loading or RyR2 refractoriness underlie the induction of calcium alternans, and we found that at the onset of altern
inactivation rates of the RyR2, calcium alternans was caused by alternation in SR calcium loading, while at low act
level of available RyR2s.

Conclusions/Significance

We have mapped cardiomyocyte beat-to-beat responses as a function of RyR2 activation and inactivation, identify
refractoriness underlie the induction of calcium alternans. A corollary of this work is that RyR2 refractoriness due t
of calcium alternans even when alternation in SR calcium load is present.

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Introduction

Despite the important role of electro-mechanical alternans in cardiac arrhythmogenesis [1], [2], its molecular origin been associated with alternation in both ionic currents and in the cytosolic calcium transient. The latter has been lir (SR) calcium uptake [3], [4], or release [4], [5], [6], [7]. Indeed, several reports [5], [7] seem to support the hypotl relationship between SR calcium load and calcium release [4]. This steep relation has been explained as a depenc receptor (RyR2) with the SR calcium bound to calsequestrin [8], thus implying a stronger release at high calcium lo

Nevertheless, cytosolic calcium alternans has been observed both in the absence and presence of concurrent fluct Recently, Shkryl et al [11] have confirmed the presence of alternans without SR calcium fluctuations and related it calcium release. This suggests that, besides calcium loading, other properties of the SR, such as activation of the the RyR2 [12], [13], recovery of the RyR2 from inactivation [12], [14], and termination of calcium release through t regulation of the beat-to-beat stability of the cytosolic calcium transient.

To address this issue, a major challenge lies in the difficulty of using experimental animal or cell models to resolve SR to the calcium transient and its beat-to-beat stability. Most often, manipulation of one parameter affects the sta of its specific contribution. We here attempt to circumvent this problem by developing a novel numerical protocol a myocyte, where we can specifically change the dynamics of SR loading and RyR2 gating, and investigate the mec alternans, under different operating conditions of the RyR2.

Methods

We used a description of a rabbit ventricular myocyte based on the model described by Shannon et al [17]. The sa the values of some parameters of the calcium dynamics were introduced. The description of the RyR2 considers t closed, and two inactivated. The nomenclature and associated reaction equations for the RyR2 are shown in Figur rates, given by the constants k_a, and k_i, were systematically changed in order to analyze their effect on the beat-to a summary of changes in the parameters). We have measured the alternans amplitude, defined as the difference i as a function of activation and inactivation rates, and for different values of the pacing period and the RyR2 recove Shannon et al [17] using the original parameters for k_a, and k_i does not give rise to calcium alternans neither at nor interval is shortened further. However, we find that changing activation and inactivation rates can produce the appe calcium alternans at 5 Hz (Figure S2 in Appendix S1), as observed in isolated rabbit cardiomyocytes [18]. Larger c generate alternans even at normal pacing rates (~3 Hz). We also considered several values of the time for RyR2 r original value), 750 ms, and 1500 ms. In most simulations we used as benchmark value τ_r = 750 ms. This value ag measured in other reports [9], [15], [19]. We checked that this benchmark value is consistent with experiments on Appendix S1).

We developed a novel numerical protocol to analyze the specific effects on calcium handling dynamics of RyR2 ac as well as SR calcium loading. In this protocol, the myocyte was stimulated at a constant pacing rate until steady s steady state response with simulations where alternations in SR Ca load, or alternations in the recovery level of R scheme is the procedure to eliminate alternations in SR Ca load or in the level of recovered RyR2s, which we achi specific details are described in the next subsection together with a discussion on how they fit in the general proble presence of calcium alternans.

Dynamic Clamping Protocols

During alternans, the intracellular cytosolic calcium transient alternates from beat to beat. Whenever this happens pre-systolic SR calcium load and the level of RyR2 ready to open (not inactivated, i.e. in state R of Figure S1 in Ap directly related with two mechanisms proposed to account for calcium alternans in the literature. One states that a cytosolic calcium alternans (calcium alternans due to SR calcium load). The other states that the level of RyR2s re experiment to discern the underlying mechanism would require eliminating the alternation in one of the variables an it does not persist, we can be confident that alternation in this variable is a necessary condition for cytosolic calciu effects this modification must be done without changing the release process, and with minimal changes in the over

While eliminating the oscillation without affecting release is unfeasible in the laboratory, the protocol we developed

myocyte model via a dynamic clamping of the variables involved. We describe first the details of the dynamic clam recovered RyR2s. Both clamping protocols can be activated separately or simultaneously. In the latter case, cytos no other intervening mechanism.

Clamping of the SR Ca Load

Figure 1 illustrates the workings of this protocol. Initially, the cell is paced at a given rate using our standard numer (see Figure 1A). Then, starting at any given beat, we change the dynamics of the sarco/endoplasmic reticulum Ca ms of diastole so that the SR calcium load achieves the same value $c^*_{SRClamp}$ before each external excitation (see equal to the maximum pre-systolic SR calcium load during the non-clamped dynamics. During the external excitatio current is used. In this way we do not affect the dynamics of calcium release and re-uptake. More importantly, the are close to their equilibrium values. As seen in Figure 1B–C the result is a clamped dynamics where the SR Ca lo where the evolution of the cytosolic calcium transient (Figure 1C) indicates if calcium alternans is affected or not b

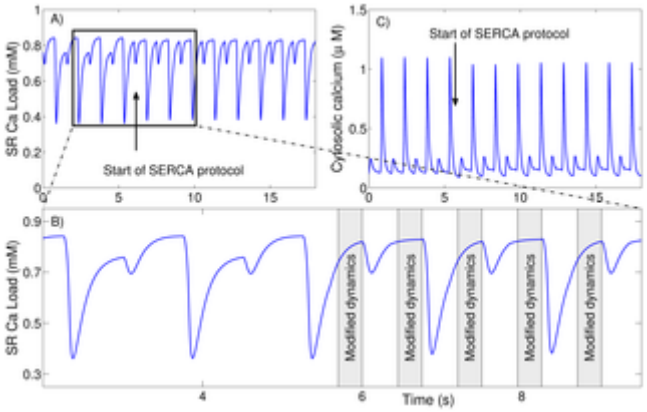

Figure 1. Dynamic protocol for eliminating oscillations in pre-systolic SR Ca load.
Panel A) indicates the moment where the protocol is activated while panel B) shows the intervals where the SE strongly in order to make the level of SR Ca load reach the same level on each beat. In this case, this level cor SR Ca load obtained before the activation of the clamping-protocol. Panel C) shows that, in this case, calcium pre-systolic SR Ca load are eliminated.
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The procedure can be summarized as testing whether calcium alternans disappears when the SERCA current is s

$$J_{SERCA} = Q_{10-SERCA} V_{\max} \frac{\left(\frac{[c]_i}{K_{mf}}\right)^H - \left(\frac{[c]_{SR}}{K_{mr}}\right)^H}{1 + \left(\frac{c_i}{K_{mf}}\right)^H + \left(\frac{[c]_{SR}}{K_{mr}}\right)^H}$$

$$\text{for} \qquad nT < t < (n+1)T - t_0 \text{ ms}$$

$$J_{SERCA} = 10 V_{\max}([c]^*_{SRClamp} - [c]_{SR})$$

$$\text{for} \qquad (n+1)T - t_0 < t < (n+1)T \quad \text{ms}$$

where typically t_0 = 150 ms (we use t_0 = 75–100 ms for T<240 ms). Equation (1) is the original SERCA uptake, wi external excitation, calcium release and first stages of the reuptake. During the last t_0 before each beat we substit which keeps pumping calcium from the cytosol until $[c]_{SR} = [c]^*_{SRClamp}$.

Clamping of RyR2 Recovery

Following the same idea of the previous clamping protocol, the clamping of RyR2 recovery is achieved changing its release (see Figure 2). These changes in the RyR2 are applied to eliminate dynamically the oscillation in the pre-s affecting calcium release and uptake. In this clamping protocol a number of RyR2 channels are disabled at a time of the recovery rate of the remaining RyR2s, so that the final recovery level of receptors before the calcium releas the total number of states read:

$$I+RI+R+O=1 \qquad \text{for} \qquad nT<t<(n+1)T-t_0$$

$$I+RI+R+O=R_{Clamp}$$
$$\text{for} \qquad (n+1)T-t_0<t<(n+1)T \quad \text{ms}$$

with the recovery rate changing form the original value to τ_r = 50 ms (k_{im} = 0.02 ms^{-1}) in the last t_0 before each ex clamped dynamics equivalent, R_{clamp} is taken to be the maximum number of pre-systolic non-inactivated channels clamping protocol is used (see Figure 2B). In effect, this means disabling a ratio of 1- R_{clamp} at time (n+1)T-t_0 and

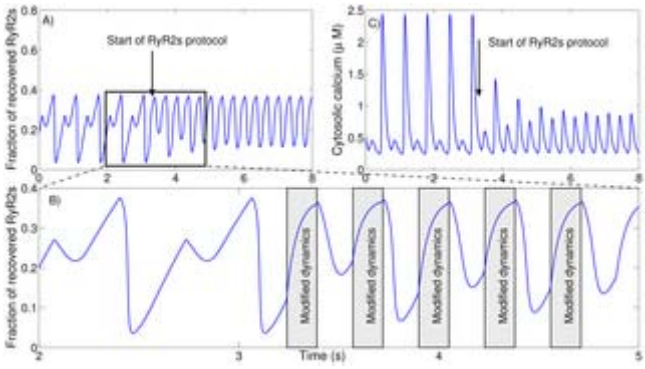

Figure 2. Dynamic protocol for eliminating oscillations in the pre-systolic level of recovered RyRs.
Panel A) indicates the moment where the protocol is activated while panel B) shows the intervals where the re same time that only a fraction of them remain active. This fraction corresponds to a recovery of 37% of the tot before the clamping-protocol is started, and it is the one we aim to reach at the end of diastole. Panel C) show eliminated when oscillations in the level of recovered RyR2s are eliminated.
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Results

Effect of RyR2 Activation and Inactivation on the Induction of Alternans

To validate the model, we first verified that changes in RyR2 activation and inactivation rates could produce alterna observed that calcium release increases with rest time, even if the content of the SR decreases (and the I_{CaL} curr this post-rest potentiation is due to a slow recovery from refractoriness of RyR2 calcium release. In the current m RyR2 from inactivation. We find that, for a recovery time of τ_r = 750 ms, the model reproduces qualitatively the po shown in Figure S6 in Appendix S1.

The original parameters in the Shannon model did not present calcium alternans at any frequency. However, Figure (k_a = 8.5 mM^{-2} ms^{-1}, k_i = 0.17 mM^{-1} ms^{-1}, or 85% and 35% of the original values), lead to calcium alternans. Alt

rate was increased from 3 Hz to 4 Hz, and thereafter became sustained at 5 Hz. Notice that changes in the RyR2 despite the fact that neither changes in the loading properties of the SERCA pump, nor in the calsequestrin (CSQN not only associated with oscillations in the SR calcium loading (c_{SR}), but also with alternations in the level of recove Subsequently, the model was used to examine how changes in the RyR2 activation-inactivation rates were able to Hz). Figure 3 shows that cytosolic calcium alternans appears when either activation or inactivation rates are dimini combinations of activation and inactivation rates, defining a boundary between uniform and alternating responses (stimulation frequency (Figure 3E). As expected, the area of alternating responses increased as the stimulation fre parameters (gray area in Figures 3B, C and D) we also observed the presence of a complex beat-to-beat behavic chaotic dynamics.

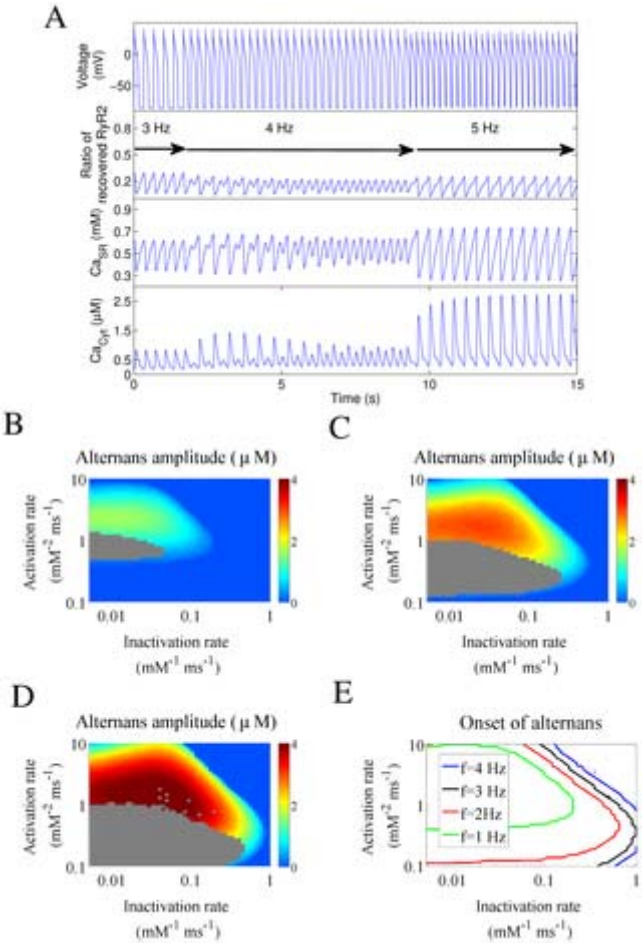

Figure 3. Slowing of RyR2 activation or inactivation induces calcium alternans at physiological pacing
A) The effect of increasing the stimulation frequency from 3 Hz to 5 Hz on trasmembrane potential (top panel), SR calcium load (lower middle panel) and cytosolic calcium (lower panel) for fixed activation and inactivation ra ms^{-1} with a recovery time from inactivation of $\tau_r = 1/k_{im}$ = 750 ms. B), C), and D) Color-code graphs showing t transient amplitude as a function of RyR2 activation and inactivation at a pacing rate of 1 Hz (B), 2 Hz (C), and RyR2 inactivation rate, while the vertical axis represents the RyR2 activation rate. The alternans amplitude, def between two consecutive beats, is given in color code with blue representing no alternans and dark red corres The gray area represents cases where a complex beat-to-beat behavior is observed, including 3:1 or 4:1 rhyth for the transition to cytosolic calcium alternans obtained with different pacing frequencies.
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To check that the observed alternations were due to instability in the calcium handling dynamics, with no significant repeated the previous simulations using an AP clamp protocol, obtaining the same results (Figure S3 in Appendix S alternans appeared due to oscillations in either SR calcium loading or RyR2 dynamics.

Mechanisms Underlying Cytosolic Calcium Alternans

In order to investigate how SR calcium load and fractional recovery of the RyR2s from inactivation contributed to c these variables and determined which of the clamping procedures was able to eliminate the cytosolic calcium alter load and of the rate of recovered RyR2 always eliminated alternans, both with current and AP clamp. Thus, in all t calcium alternans is related to either SR Ca load, recovery of the RyR2 from inactivation, or both.

Figure 4A shows an example where only a clamping of the SR calcium load eliminated alternans, demonstrating th necessary for the induction of alternans. Figure 4B shows an example where calcium alternans disappears only wl and thus the responsible mechanism is alternation in the number of RyR2 that are recovered from inactivation. Figu either variable eliminates calcium alternans or neither of them alone does. Thus, in Figure 4C both mechanisms are 4D either of them by itself is able to maintain it, without being necessary the presence of the other.

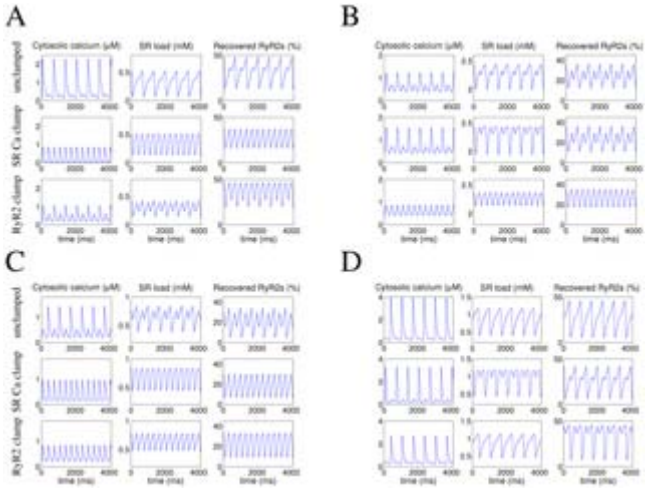

Figure 4. Contribution of SR calcium load and recovery of RyR2 from inactivation to the induction of c
doi:10.1371/journal.pone.0055042.g004

Each of these examples was obtained with different combinations of activation and inactivation rates. To determine for any given combination of the RyR2 activation and inactivation rates, we repeated the simulations shown in Figu or the fraction of recovered RyR2s (Figure 5C). When the SR calcium load was clamped (Figure 5B), the boundar values of activation or inactivation, but there was still a large area where alternans is present. This indicated that r sustain alternans in that region. On the other hand, when the fraction of recovered RyR2s was clamped (Figure 5C large area. Therefore, combining Figures 5A, B, and C allowed us to identify the regions where (see Table 1): 1) a mechanism underlying calcium alternans (region “L”); 2) recovery of the RyR2 from inactivation is the responsible r necessary (region “R+L”); 4) either mechanism is able to sustain alternans (region “R, L”). Figure 5D shows how t activation and inactivation rates for a pacing frequency of 3 Hz.

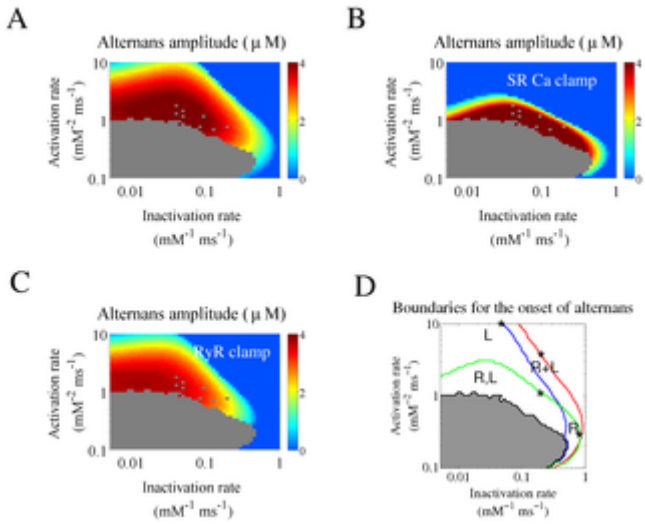

Figure 5. Mechanism underlying the onset of alternans at different activation and inactivation rates.
A) Color-code graph showing the amplitude of the cytosolic alternans at 3 Hz. Blue indicates the absence of alt
The same simulations as in A) but with SR Ca loading clamped at presystolic values. C) The same simulations
RyRs clamped at presystolic values. D) Lines denoting the onset of alternans under: normal (un-clamped) conc
line), and clamped fraction of recovered RyRs (blue line). The gray area represents the region with irregular be
lines delimitate the regions where alternations in SR calcium load ("L") and RyR2 recovery ("R") are responsibl
region where alternations in either recovery of RyR2s from inactivation or SR Ca load are capable of maintainir
both mechanisms to sustain cytosolic calcium alternans. The four asterisks correspond to the four examples sh
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		Mechanism			
		"R"	"L"	"R+L"	"R, L"
Clamping protocol	SR Clamping	Alternans Persists	Alternans Disappears	Alternans Disappears	Alternans Persists
	RyR2 Clamping	Alternans Disappears	Alternans Persists	Alternans Disappears	Alternans Persists

"R" stands for alternans due to alternation in RyR2 recovery from inactivation, "L" stands for alternans due to alternation in SR Ca load, "R+L" stands for alternans that require both oscillations in the recovery of RyR2s and in SR Ca load. Finally, "R,L" stands for alternans where both mechanisms contribute but either can sustain it. The case where both protocols were applied at the same time is not shown since, in all cases, alternans disappeared.
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Table 1. Mechanisms of calcium alternans.
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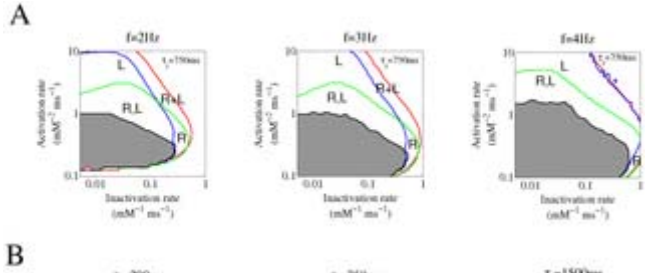
To further understand the presence of alternans when SR load does not alternate, we considered an idealized situ
action potential clamp, and 2) the SR calcium and 3) the subsarcolemmal calcium were fixed at a constant concen
still appears, the RyR2 dynamics is its only possible source. From a mathematical analysis of this case (see Secti
presence of an instability that gives rise to alternans, through a period-doubling bifurcation (Figure S4 in Appendix
dynamics and requires a stimulation period shorter than its recovery time from inactivation (Figure S5 in Appendix

We then investigated how the stimulation frequency affects the relative relevance of the different mechanisms, rec
3 Hz and 4 Hz) and the results are summarized in Figure 6A.



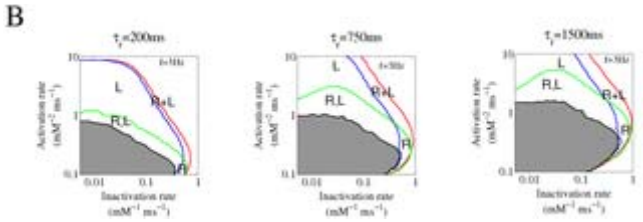


Figure 6. Mechanisms responsible for the onset of alternans for different pacing rates and RyR2 recov
The limits for the onset of alternans are shown as in Figure 5D (reproduced here in the central panels), for diffe τ_r = 750 ms). B) The limits for the onset of alternans for different values of the RyR2 recovery time τ_r: 200 ms, Hz).
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Effect of Changes in the Recovery Time of the RyR2 from Inactivation

Figure 6B shows that the boundaries of calcium alternans enlarge as the time for recovery of the RyR2 from inacti value of 750 ms, and further to 1500 ms. To expand this analysis to different frequencies, four representative sets were selected, corresponding to regions “R”, “L”, “R, L”, and “R+L”. Figure 7 shows how the stimulation frequency alternation. Notice that for the four sets of parameters considered, increasing the stimulation rate, the onset of alte frequency) under conditions where both mechanisms are required (“R+L”). Similarly, diminishing the RyR2 recover recovery from inactivation contributed to the maintenance of calcium alternans. Finally, the contribution of SR calciu became more predominant at high frequencies.

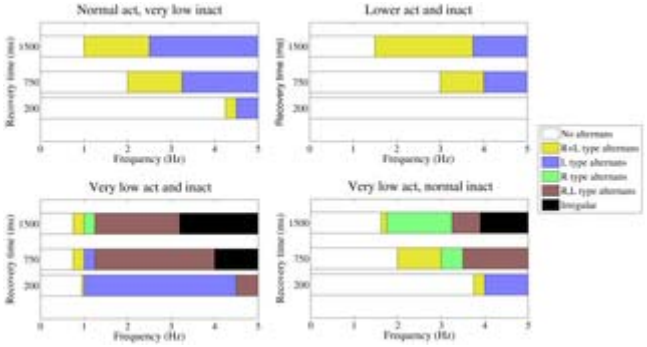


Figure 7. Mechanism underlying the onset of alternans at different pacing frequencies and RyR2 recov
. The four panels illustrate how the mechanism underlying the induction of cytosolic calcium alternans varies wit from inactivation. Each panel has three rows of color bars, which indicates the responsible mechanism for the i frequencies. The top bar represents slow RyR2 recovery (τ_r = 1500 ms), the middle bar intermediate RyR2 rec RyR2 recovery from inactivation (τ_r = 200 ms). Colors green, purple, yellow, and brown correspond, respective 1. Black indicates frequencies where irregular behavior is present. The parameters for activation and inactivatio = 0.05 mM^{-1} ms^{-1}, right: k_a = 3.5 mM^{-2} ms^{-1}, k_i = 0.2 mM^{-1} ms^{-1}; lower panels, left: k_a = 1.0 mM^{-2} ms^{-1}, k_i = = 0.5 mM^{-1} ms^{-1}.
doi:10.1371/journal.pone.0055042.g007

Discussion

Main Findings

The present study has used a mathematical myocyte model and a numerical clamping protocol to map beat-to-bea function of RyR2 activation and inactivation as well as the identification of domains where SR calcium load and/or l induction of calcium alternans. This approach makes it possible to identify transition zones where one predominant

characterization of how the transition zones depend on the stimulation frequency, SR calcium load and the RyR2 re predict how mutations or drugs that affect RyR2 gating properties will modify the beat-to-beat stability of calcium l demonstrates that even when experimental data shows concurrent alternations in calcium load and the cytosolic ca that alternation in calcium load is the underlying mechanism.

Validation and Limitations of the Model

The current approach used a validated rabbit ventricular myocyte model [17] that incorporates realistic features of reproduced calcium dynamics under steady-state conditions. Taking into account that elevation of the stimulation fr cytosolic calcium transient upon elevation of the stimulation frequency [4], [7], [9], we identified modifications of the activation time, inactivation time, and/or the recovery time from inactivation) that were necessary for the model to

For the present analysis activation times ranged from 0.01 to 1x the values used by Shannon et al [17]. These valu alternans could be induced, but it has also been shown that mutations in the TM10 region of the RyR2 can in fact r as much as 1,000 fold less than the wild type [20]. For inactivation times, we used values that ranged from 0.01 to which is also consistent with reports showing that the failure to completely terminate Ca^{2+} release following channe loss of Ca^{2+}-dependent inactivation (8- to 10-fold) [21]. The present RyR2 model includes inactivation processes c release. This is contrary to the behavior in single RyR2 dynamics, where refractoriness of gating has never been c to be significant on a beat-to-beat time scale. Thus, the RyR2 dynamics in the present model must be understood behavior of several RyR2s or clusters of RyRs that generate calcium sparks, where long time refractoriness has b

The model used does not consider stochastic variations among calcium release units (CaRUs), and can therefore model does not present calcium waves, although complex or chaotic beat-to-beat patterns are observed (gray are represent a serious limitation when analyzing synchronized responses where calcium waves are not present.

Besides the specific ventricular myocyte model used here, the protocol employed to uncover the mechanism behin any other whole cell cardiomyocyte model.

Effect of RyR2 Activation and Inactivation on the Induction of Calcium Alternans

The two-dimensional mapping of the beat-to-beat response as a function of RyR2 activation and inactivation show inactivation leads to the induction of calcium alternans. Moreover, it showed that elevation of the stimulation freque calcium alternans towards faster activation and/or inactivation rates. In this context, low levels of RyR2 activation h alternans in experiments where the RyR2 open probability (P_o) was decreased with tetracaine or intracellular acidi variability in the Ca^{2+} transient [6]. Considering that decreased RyR2 open probability may arise from either slowe present model confirms that slower RyR2 activation does indeed promote calcium alternans but shows that faster alternans, suggesting that tetracaine and intracellular acidification likely decreases the RyR2 open probability by sl

Role of SR Calcium Load and RyR2 Recovery from Inactivation on the Induction o

Our results show that slowing of inactivation leads to calcium alternans, which is abolished when SR calcium loadi oscillations are necessary for alternans in this case. Indeed, alternation in SR Ca load is a widely accepted explan on a steep relationship between SR calcium loading and Ca^{2+} release that makes any small difference in loading in feedback mechanism (see Shiferaw et al [4]). Physiologically, the origin of this steep relationship could be an effec [12], [22] or it could be caused by desynchronized calcium release in the form of calcium waves, that mark an abru load-release relationship [23]. Here, we reproduce, by changing RyR2 refractoriness, alternations in cytosolic calc sarcoplasmic reticulum calcium fluctuations. Very low inactivation rates correspond, effectively, to situations where RyR2 which transit to inactivation is very low. This leads to an effective two-state model of RyR2, which presents a SR load and release. Alternans due to SR Ca load has also been obtained numerically by Restrepo et al [8] using and two open states.

Calcium alternans is also induced by a slowing of RyR2 activation, if inactivation is non-negligible. In this case, alte not by clamping SR Ca load, indicating that incomplete RyR2 recovery is the underlying mechanism. The physiolog the results of the post-rest protocol, where we observe that the calcium transient increases for increasing rest tim S6 in Appendix S1). These simulations also agree with the experimental results by Picht et al [9], linking calcium al post-rest potentiation. Together, this suggests that the mechanism underlying alternans termed “R” in our simulatio al. Alternatively, cytosolic calcium alternans at constant diastolic values of SR calcium loading has been explained

involving RyR2 recovery, recruitment and randomness of the calcium release units (CaRUs). Their model produces different parts of the cells, which is in accordance with results from calcium overloaded rat ventricular myocytes by shown in human atrial myocytes with normal SR calcium load that calcium release is typically synchronized during

In concordance with recent experiments [11], we also show that although oscillations in SR Ca load are present, th alternans. In our analysis of the model, when the SR is loaded above a certain threshold all RyR2s are activated b RyR2 are filled. Oscillations in c_{SR} can therefore not drive calcium alternans. By contrast, oscillations in RyR2 refra alternans. Inactivation is dependent on the calcium concentration at the dyadic space, so that a larger calcium dep RyR2 channels, which in turn may cause incomplete RyR2 recovery at fast pacing rates. Under such conditions, th released from the SR and the fraction of the recovered RyR2s [26]. This situation is favored when both RyR2 activ

We have shown that is indeed the case considering a situation where both the SR calcium and subsarcolemma ca Appendix S1). Under this condition the concentration of calcium in the dyadic space increases when the L-type cal release of calcium from the SR. Therefore, the presence of alternans can only be explained because of nonlinearit the RyR2. A full analysis of this nonlinearity, shows that RyR2 dynamics can indeed lead to calcium alternans (Figu

Physiological and Pathophysiological Predictions of the Model

A number of studies have reported on associations between cardiac rhythm disturbances and abnormal SR functio state of phospholamban and the RyR2 that are known to modulate SR calcium loading [27] and RyR2 opening [25 reports linking heart failure [30], CPVT [31] and atrial fibrillation to increased phosphorylation and/or calcium releas [32]. An increase in RyR2 opening could result from longer and/or more frequent RyR2 opening. Longer opening ir and/or faster RyR2 recovery from inactivation, while more frequent opening would require faster RyR2 activation a model shows that faster RyR2 activation as well as faster RyR2 recovery prevents the induction of calcium alterna towards higher stimulation frequencies, making them unlikely to be mechanisms underlying the induction of calcium our analysis, it has recently been shown that drugs that increase the frequency of RyR2 openings but decrease th effects [33], [34].

By contrast, our results show that a slowing of RyR2 inactivation would promote calcium alternans and lower the b induced suggesting that these arrhythmias are likely associated with a slowing of RyR2 inactivation. In accordance RyR2 calcium release has been reported in patients prone to arrhythmia [35]. Consequently, pharmacological inter increasing RyR2 inactivation are expected to be antiarrhythmic by preventing both spontaneous SR calcium releas analysis of the model also predicts that antiarrhythmic candidates such as tetracaine that prevent RyR2 opening b because they favor the induction of calcium alternans. In line with this, it has been shown experimentally that tetrac beat-to-beat responses at lower stimulation frequencies in human atrial myocytes [25], [36].

Another field gaining increasing attention is genetic mutations linked to abnormal RyR2 function or SR calcium load mutations causing SR overload or calcium leak are likely to be arrhythmogenic by promoting calcium release-induc out above, the present model predicts that mutations that increase RyR2 open probability by increasing RyR2 acti expected to prevent the induction of calcium alternans.

On the other hand, our results also suggest that mutations which decrease RyR2 open probability by reducing RyR induce calcium alternans at lower beating rates. In accordance with this prediction, the first mutation in the RyR2 a which dramatically reduces RyR2 opening, was recently described and shown to be associated with a strong redu Together, this shows that the present model may be useful to understand and predict the relationship between mo and rate-dependent beat-to-beat changes in the intracellular calcium transient in isolated cardiomyocytes.

Conclusion

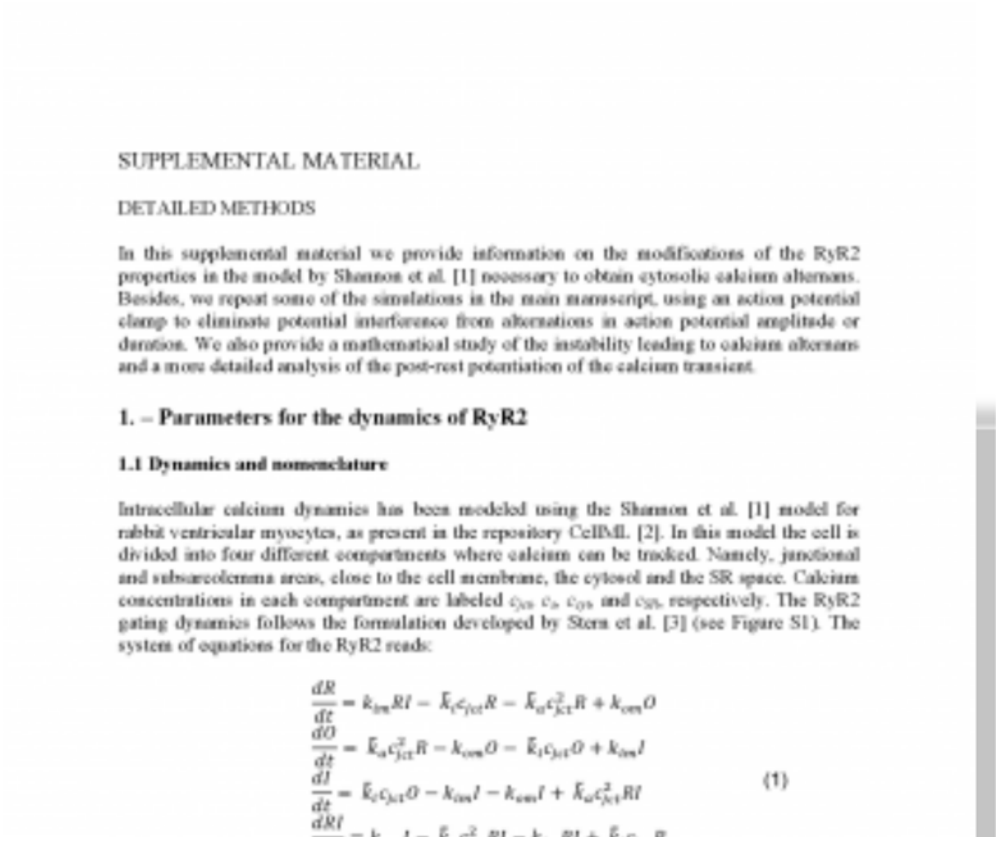
The present study has used a well characterized rabbit numerical ventricular myocyte model and a dynamic clamp fundamental RyR2 properties such as activation, inactivation, and recovery from inactivation as well as SR calcium dependent induction of cytosolic calcium alternans. This approach allows a mapping of the beat-to-beat response well as the identification of domains where SR calcium load and/or RyR2 recovery from inactivation contribute to th identification of transition zones where one predominant mechanism is substituted by another, and a characterizati stimulation frequency or the RyR2 recovery time. Importantly, the developed clamping protocols can also be used cardiac myocyte models.

A consequence of our study relevant to the analysis of other cardiac cell types is that even when experimental dat

and the cytosolic calcium transient, this does not necessarily imply that alternation in calcium load is the underlying

Supporting Information

Appendix_S1.pdf



SUPPLEMENTAL MATERIAL

DETAILED METHODS

In this supplemental material we provide information on the modifications of the RyR2 properties in the model by Shannon et al. [1] necessary to obtain cytosolic calcium alternans. Besides, we repeat some of the simulations in the main manuscript, using an action potential clamp to eliminate potential interference from alternations in action potential amplitude or duration. We also provide a mathematical study of the instability leading to calcium alternans and a more detailed analysis of the post-rest potentiation of the calcium transient.

1. – Parameters for the dynamics of RyR2

1.1 Dynamics and nomenclature

Intracellular calcium dynamics has been modeled using the Shannon et al. [1] model for rabbit ventricular myocytes, as present in the repository CellML [2]. In this model the cell is divided into four different compartments where calcium can be tracked. Namely, junctional and subsarcolemma areas, close to the cell membrane, the cytosol and the SR space. Calcium concentrations in each compartment are labeled c_{jct}, c_s, c_{cyt} and c_{SR}, respectively. The RyR2 gating dynamics follows the formulation developed by Stern et al. [3] (see Figure S1). The system of equations for the RyR2 reads:

$$\begin{aligned}
\frac{dR}{dt} &= k_{inv}RI - \bar{k}_i c_{jct}R - \bar{k}_a c_{jct}^2 R + k_{cov}O \\
\frac{dO}{dt} &= \bar{k}_a c_{jct}^2 R - k_{cov}O - \bar{k}_i c_{jct}O + k_{inv}I \\
\frac{dI}{dt} &= \bar{k}_i c_{jct}O - k_{inv}I - k_{cov}I + \bar{k}_a c_{jct}^2 RI \qquad (1) \\
\frac{dRI}{dt} &= \cdots
\end{aligned}$$

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Supplemental material with further information on the modifications of the RyR2 properties in the model by Shannon et al. necessary to obtain cytosolic calcium alternans. It also includes some extra simulations, using an action potential clamp to eliminate potential interference from alternations in action potential amplitude or duration. Finally, it provides a mathematical study of the instability leading to calcium alternans and a more detailed analysis of the post-rest potentiation of the calcium transient. The appendix includes the following sections and figures: 1. – Parameters for the dynamics of RyR2 (with Figures S1, S2 and S3). 2.- Return map analysis of calcium alternans at constant SR load (with Figures S4 and S5). 3. – Restitution of calcium release (with Figure S6).

Appendix S1.

Supplemental material with further information on the modifications of the RyR2 properties in the model b calcium alternans. It also includes some extra simulations, using an action potential clamp to eliminate potential ir amplitude or duration. Finally, it provides a mathematical study of the instability leading to calcium alternans and a of the calcium transient. The appendix includes the following sections and figures: 1. – Parameters for the dynamic Return map analysis of calcium alternans at constant SR load (with Figures S4 and S5). 3. – Restitution of calcium

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(PDF)

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Author Contributions

Prepared figures: EA-L BE Edited and revised manuscript: EA-L IRC AP JC LH-M BE.. Conceived and designed t
Performed the experiments: EA-L BE. Analyzed the data: EA-L IRC AP JC LH-M BE. Wrote the paper: EA-L LH-M

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