1. Introduction

In normal ventricular myocytes, the transverse-tubule (TT) network exists to facilitate effective excitation-contraction coupling by allowing synchronous calcium (Ca) release from the deep within the interior as well as the surface of the cell [1,2]. Voltage-gated L-type Ca channels (LCCs) are distributed in clusters in the TT membrane in close proximity to clusters of ryanodine receptor (RyR) channels in the sarcoplasmic reticulum (SR) membrane, forming a Ca release unit (CRU) network. Ca entry from the opening of one or more LCCs in a CRU can trigger the opening of its associated RyRs to release SR Ca into the cytosol via Ca-induced Ca release (CICR). During an action potential, the activation of LCCs in the TT network allows simultaneous Ca release of the CRUs throughout the cell and thus facilitates synchronous contraction of the myofilaments, a key feature promoting effective excitation-contraction coupling. In heart failure (HF), the TT network becomes disorganized and disrupted [3–11], causing some CRUs to lose their corresponding LCCs and resulting in so-called orphaned RyR clusters [4]. Lacking associated LCCs, the RyRs in the orphaned RyR clusters cannot be directly activated by LCCs, but have a probability of being activated later by Ca released from the neighboring CRUs. This leads to dysynchronous Ca release and a reduced Ca transient amplitude [5,8], which impairs contractile function. Besides causing dysynchronous Ca release, TT disruption can also have consequences on the myocyte’s action potential properties [10,12], which may contribute to arrhythmogenesis [13,14].

In addition to TT disruption, HF remodeling involves many other proteins [15–18], including increased RyR leakiness, down-regulated sarcoplasmic/endoplasmic reticulum Ca ATPase (SERCA), and altered ionic currents, as well as structural changes, which depend on the stage of HF development. In addition to contractile dysfunction, these changes also promote arrhythmogenesis in HF, notably pulsus alternans and T-wave alternans (TWA), which have been known for more than a century to increase arrhythmia risk [19,20]. TWA is currently used clinically as a risk prognosticator for ventricular arrhythmias in patients with HF [21–23]. Experimental studies have shown that pacing-induced HF remodeling promotes action potential duration (APD) and
Ca alternans [24–27], with Ca alternans being the primary origin. Two mechanisms of Ca alternans have been described and analyzed both experimentally and theoretically: 1) SR Ca load-dependent alternans, in which SR Ca content alternates in phase with cytoplasmic free Ca, requiring a steep SR Ca release-load relationship (or fractional SR Ca release curve) [28–34]; 2) refractoriness-dependent alternans, in which refractoriness of RyRs is required for Ca to alternate, while SR Ca load alternans is not required [35–40]. However, how TT disruption interacts with other remodeling factors to promote Ca alternans in HF, and which specific mechanisms are involved, are still poorly understood.

Accordingly, in this study, we investigated the effects of TT disruption and other HF remodeling factors on the susceptibility to Ca alternans in computer simulations, using a ventricular myocyte model with detailed spatiotemporal Ca cycling. The model contains a CRU network of spatially-distributed LCC and RyR clusters, in which TT disruption was simulated by random removal of LCC clusters and redistribution of the corresponding LCCs to other CRUs in the cell. Since the electrophysiological remodeling in HF is complex and depends on the stage of HF as well as species, for simplicity, we carried out simulations under the following conditions: 1) normal Ca cycling proteins; 2) remodeled Ca cycling proteins; and 3) remodeled Ca cycling proteins which a percentage (pLCC) of LCC clusters were randomly removed, and other remodeled ionic currents. We show that: 1) for normal SERCA activity, TT disruption, and CRU refractoriness, and CRU recruitment interact to cause alternans, as described by the 3R theory developed by us previously [37,41]; 2) for down-regulated SERCA, Ca alternans is caused by a steep SR load-release relationship. Alternans due to this mechanism can occur at very slow pacing rates. Increased RyR leakiness and TT disruption shift and steepen the fractional SR Ca release curve to promote alternans. We conclude that the mechanisms of Ca alternans for normal and down-regulated SERCA are different, and TT disruption promotes Ca alternans by both mechanisms, which may contribute to arrhythmogenic alternans in different stages of HF.

2. Methods

2.1. Ventricular cell model

The ventricular cell model is an action potential model with detailed spatiotemporal Ca cycling which includes a diffusively-coupled network of 100x20x10 CRUs, forming a three-dimensional cell (Fig. 1A). Details of the model were presented in our previous publication [42], and the parameters were the same unless stated in the present study. Briefly, each CRU contains a network space (NSR), a junctional SR space (JSR), a dyadic space (DS), and a cytosolic space. Ca diffuses freely between the NSR and the JSR, and between the DS and the cytosolic space. Under normal conditions, each CRU contains one cluster of 10 LCCs and one cluster of 100 RyRs. Ca enters the DS via the LCCs, which were simulated with stochastic Markov transitions. Ca is released from the JSR to DS via the RyRs which were also simulated by stochastic Markov transitions. Ca is extruded from cytosol via Na-Ca exchange (NCX) and taken up from cytosol via the SERCA pump. The CRUs are locally coupled by Ca diffusion in the cytosol and in the NSR. Fig. 1B shows an action potential and a line scan of cytosolic Ca of the normal control model at a pacing cycle length (PCL) of 500 ms. The cell model was periodically paced with this normal action potential wave form (action potential clamp) for all simulations except the ones in Fig. 7.

2.2. TT disruption

TT disruption was modeled by removing LCC clusters from the CRU network, causing the corresponding RyR clusters to become orphaned. Since no quantitative data is available on TT disruption, we randomly chose CRUs with a probability pLCC and removed their corresponding LCC clusters, i.e., pLCC is the percentage of LCC clusters removed. Experimental studies have shown that the whole-cell ICa,L in HF is either reduced or unchanged [15,18]. In most of the simulations, we retained the same whole-cell ICa,L by randomly re-distributing the removed LCCs back into the CRUs with LCC clusters. Therefore, with the exception of the simulations in Fig. 3, TT disruption for all other simulations in which a percentage (pLCC) of LCC clusters were randomly removed, the total number of LCCs remained constant.

Fig. 1. Computational model. A. Schematic plot of the CRU network model. The full model contains 10x20x100 CRUs. A CRU contains a network SR (NSR) space, a junctional SR (JSR) space, a dyadic space (DS), and a cytosolic (Cyto) space. These spaces are coupled via SR Ca release (Jrel), uptake (Jup), and Ca diffusion. The CRU network is coupled via Ca diffusion in cytosol and NSR. B. An action potential and a line scan of cytosolic free Ca under control conditions at PCL = 500 ms. We set [Na] = 9 mM as normal [Na]. This action potential was used as the voltage wave form for all simulations under action potential clamp conditions.

M. Nivala et al. / Journal of Molecular and Cellular Cardiology 79 (2015) 32–41
2.3. Other HF remodeling

HF remodeling is a complex process which depends on the stage and etiology of HF. Here we choose a set of parameters to simulate other HF remodeling alterations largely based on the two references [17,43] as follows: 1) RyR leakiness was increased by increasing the transition rate from the closed state to the open state by 30%; 2) the maximum SERCA activity was reduced by 50% and $K_d$ was reduced from 1 μM to 0.5 μM to simulate increased phospholamban phosphorylation [44]; 3) $I_{NCX}$ was doubled; and 4) $I_{Ks}$, $I_{Kr}$, and $I_{K1}$ were reduced by 50%. In this study, intracellular sodium concentration ($[\text{Na}^+]_i$) was used as a parameter instead of a variable, which is elevated in HF.

2.4. Numerical methods

The details of the computational methods have been presented previously [42]. Briefly, the NSR and cytosolic space were discretized into three-dimensional spatial grids using a 0.2x0.2x0.2 μm³ spatial resolution, such that each CRU contains 250 computational voxels, a JSR, and a DS. Therefore, a 20,000-CRU cell model contains more than 2 million randomly simulated ion channels and 5 million computational voxels. The equations were simulated using an operator splitting method with a time step of 0.01 ms. Although our code can simulate 1 s of heart time in 10 minutes of real time with our advanced computational methods [42], it is still computationally expensive for large-scale simulations. To perform systematic studies, we used a smaller CRU network which contains 20x10x5 CRUs (for simulations in Figs. 4 and 5). As we have shown before [38] (and confirmed in present study), the smaller grid gives almost the same result as the full model with slightly larger random fluctuations. All computations were performed on an Intel Xeon 2.53 GHz processor using Graphical Processing Unit parallel computing with an NVIDIA Tesla C2050.

3. Results

3.1. Effects of TT disruption on the Ca transient, SR load, and fractional SR Ca release

Figs. 2A–C compare SR Ca concentration ($[\text{Ca}^+]_{SR}$), the whole-cell free cytosolic Ca ($[\text{Ca}^+]_i$) and line scans of free cytosolic Ca for three conditions: 1) normal control (panel A); 2) RyR, SERCA and NCX remodeled without TT disruption (panel B); and 3) RyR, SERCA, and NCX remodeled with TT disruption (panel C). Remodeling without TT disruption reduced SR Ca load, increased the rise time of $[\text{Ca}^+]_i$, reduced

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** Effects of HF remodeling on the Ca transient, SR load, and fractional SR Ca release. Shown are whole-cell SR Ca, cytosolic free Ca, and a line scan of the cytosolic free Ca. SR load and cytosolic Ca time-to-peak are listed. A) Normal control. B) RyR, SERCA and NCX remodeled without TT disruption. C) RyR, SERCA and NCX remodeled with TT disruption ($\text{PCC} = 30\%$). PCL = 500 ms and $[\text{Na}^+]_i = 9$ mM. D) Effects of remodeling and TT disruption on fractional SR Ca release curves under the same conditions as in A-C. The fractional release curves were calculated as described in a previous study [37].
peak [Ca], and made Ca release more dysynchronous (see the line scans). The addition of TT disruption exacerbated the alterations to the Ca transient and dysynonymus Ca release, but modestly increased the SR Ca load. The reason is straightforward: the efficiency of SR Ca release was reduced by orphand RyR clusters, such that more Ca was retained in the SR and less was extruded by NCX, increasing the SR Ca load. Therefore, while TT disruption enhances dysynonymus Ca release, it can also increase SR load if the whole-cell ICa,L is not reduced.

Fig. 3A shows that 30% of the LCC clusters were removed, but their LCCs re-distributed to the other CRUs with LCC clusters such that the total number of LCCs remain the same. [Na]i = 14 mM. 30% LCC clusters removed (black traces in Fig. 3), despite a doubling of peak [Ca], (compare to Fig. 1B).

As shown in our previous studies [37,38], reducing ICa,L promotes Ca alternans by reducing the number of primary sparks activated by LCC openings, thereby making more CRUs available to be recruited as secondary (spark-induced) sparks. By reducing the single channel conductance gLCC by 30%, peak ICa,L decreased from 6 pA/pF to 4 pA/pF, and Ca alternans now occurred at the same PCL = 500 ms (purple traces in Fig. 3A). In the second case, 30% of the LCC clusters were randomly removed from the myocyte (resulting in 30% of the RyR clusters being orphaned), which also caused a 30% reduction in whole-cell ICa,L and promoted Ca alternans (purple traces in Fig. 3B). In the third case, instead of removing the LCCs from the myocytes, these (30%) LCCs were randomly redistributed to other CRUs (so that the total number of LCCs remained the same and the whole cell ICa,L was not decreased, and in fact became slightly larger due to a weaker Ca-dependent inactivation of the LCCs that were no longer sensing the higher Ca in dyadic space), Ca alternans still developed (purple traces in Fig. 3C). These findings show that not only is the amplitude of whole-cell ICa,L important for Ca alternans, but also the spatial distribution of LCCs.

To systematically study the effects of the spatial distribution of LCCs on Ca alternans, we carried out simulations using different levels of Ca load (by changing [Na]i) and varying the percentage of TT disruption (with total LCCs remaining the same by redistributing the LCCs from the removed LCC clusters to other CRUs). The results at a fixed PCL = 500 ms are shown in Fig. 4A. With no TT disruption or [Na]i < 10 mM, alternans did not occur. Both [Na]i elevation and TT disruption were required to induce Ca alternans at this PCL. The amplitude of alternans increased as [Na]i or the percentage of TT disruption increased. Fig. 4B shows that for a fixed [Na]i of 11 mM, increasing the percentage of TT disruption promoted alternans and increased SR load.

M. Nivala et al. / Journal of Molecular and Cellular Cardiology 79 (2015) 32–41
The dependence of Ca alternans on PCL is shown in Fig. 4C. For \([\text{Na}]_i = 11 \text{ mM}\) and \(p\text{LCC} = 30\%\), alternans started between PCL = 800 ms and 700 ms, and its amplitude increased together with SR load as PCL was decreased further. SR load alternated together with peak \([\text{Ca}]_i\). However, if \([\text{Ca}]_\text{SR}\) was clamped at a constant load (700 \(\mu\text{M}\)) and other conditions were the same as in Fig. 4C, alternans still occurred, although its onset started at a shorter PCL (between 500 ms and 400 ms), as shown in Fig. 4D. Since alternans occurred even when SR Ca was clamped, this indicates that refractoriness of SR Ca release was primarily responsible for alternans. However, SR load alternans also amplified Ca alternans under these conditions, since the onset of alternans occurred at a longer PCL when the SR load was allowed to vary freely.

### 3.3. Effects of TT disruption on Ca alternans when SERCA is down-regulated

We next studied the condition in which SERCA is down-regulated with increased RyR leakiness and \(I_{\text{NCX}}\) as described in Methods. Fig. 5A shows how \([\text{Na}]_i\) and the percentage of TT disruption affected the alternans amplitude (\(\Delta\text{Ca}_{\text{ALT}}\)). TT disruption promoted Ca alternans, but, unlike the case in Fig. 4A, TT disruption was not obligatory, since at 0\% TT disruption, Ca alternans still occurred, albeit at a higher \([\text{Na}]_i\).

Fig. 5B shows peak \([\text{Ca}]_i\) and SR load versus \(p\text{LCC}\) for \([\text{Na}]_i = 10 \text{ mM}\) at PCL = 500 ms. Fig. 5C shows peak \([\text{Ca}]_i\) and SR load at different PCL for \(p\text{LCC} = 30\%\) and \([\text{Na}]_i = 10 \text{ mM}\). Under these conditions, alternans occurred at very slow pacing rates between PCL = 4 s and 5 s. As PCL shortened, the amplitude of both the Ca transient and \(\Delta\text{Ca}_{\text{ALT}}\) decreased modestly. SR load also decreased until PCL = 1 s, beyond which the SR Ca load increased. Alternans disappeared when SR Ca was clamped (Fig. 5D), indicating that Ca alternans depended on SR load alternans. Moreover, at these slow pacing rates, the SR and RyRs were fully recovered. Therefore, the refractoriness of SR Ca release due to SR refilling or RyR recovery played no role here. Interestingly, if the magnitude of the SERCA was restored to the normal value, the onset of alternans now occurred at a much shorter PCL (Fig. 5E), in the same range as in Fig. 4C, indicating the importance of decreased SERCA pump activity in promoting alternans. Note that Ca alternans at slow heart rates (\(\leq 0.5 \text{ Hz}\)) have been observed in experimental studies [29,33,35].

Fig. 6 compares traces of \([\text{Ca}]_i\), \([\text{Ca}]_\text{SR}\), \(I_{\text{NCX}}, I_{\text{CaL}}\) and the percentage of RyR available for opening during alternans when RyR, SERCA and NCX properties were normal (panel A) or remodeled (panel B). A major difference is that for normal SERCA activity, Ca uptake by the SR was much greater following the large release beat. However, when SERCA was down-regulated, Ca uptake by the SR was less following the large release beat than that following the small release beat (see Discussion).

### 3.4. Effects of HF remodeling on both APD and Ca alternans

Besides remodeling of Ca cycling proteins, many ion channels are also remodeled in HF. Therefore, we also simulated a third condition in which we added these ionic current alterations (see Methods) to the Ca cycling protein remodeling to study the interaction between voltage and Ca on alternans. We paced the model with voltage running freely (equivalent to current clamp conditions). Under the normal control condition, decreasing the PCL from 500 to 200 ms did not induce alternans. When Ca cycling properties and ionic currents were modified to simulate HF remodeling, with 30\% TT disruption and elevated \([\text{Na}]_i\) from 9 to 10 \(\text{ mM}\), APD prolonged from 160 ms (Fig. 1B) to 230 ms.
(Fig. 7A). APD and Ca alternans now both occurred at very slow heart rates, beginning at PCL = 5 s. If, instead of allowing voltage to run freely, we paced the myocyte with the action potential waveform corresponding to that immediately before the onset of alternans, Ca alternans still occurred at the same PCL with roughly the same magnitude (open circles in Fig. 7B). This indicates that alternans originates primarily from the Ca cycling system in this model. Moreover, the lower SERCA activity is the major cause of alternans at slow rates, based on the simulations above.

4. Discussion

TT disruption is one of the remodeling consequences of HF known to contribute to dys synchronous Ca release and impair excitation-contraction coupling. In a recent study [14], Hong et al. showed that loss of TTs also increased the propensity to arrhythmias in mouse hearts. However, the mechanistic link between TT disruption and arrhythmias is not well-understood [13]. In this study, we used computer modeling to investigate this issue and show that TT disruption plays an important role in promoting Ca alternans, a precursor which predisposes the heart to spatially discordant APD alternans and initiation of reentrant arrhythmias [46].

4.1. Mechanisms of TT disruption promoting Ca alternans

Our findings indicate that TT disruption per se does not cause Ca alternans, but promotes Ca alternans in combination with other changes. Two mechanisms of Ca alternans have been described [41,47]. The first mechanism is SR load-dependent, which was rigorously analyzed in theoretical studies [28,30] and supported by evidence from experiments [29,32,33]. This mechanism depends on a steep SR release-load relationship and reduced SERCA activity which work synergistically to promote Ca alternans, which is mathematically described as [30]:

\[ l_{k+1} = l_k - g(l_k) + h(c_{k+1}^c) \]

where \( l_k \) is the SR load of the previous paced beat and \( l_{k+1} \) is that of the present beat, \( g(l_k) \) is the fractional SR Ca release and \( h(c_{k+1}^c) \) is the Ca taken up by the SR, with \( c_{k+1}^c \) being the peak [Ca], of the present beat. Eq. (1) predicts that alternans occurs when

\[ (g' - 1)(1 - h') > 1 \]

where \( g' \) and \( h' \) are the slopes of the two functions at the steady state, respectively. Therefore, alternans is promoted by a steep fractional SR release curve (large \( g' \)) and/or a shallow SR uptake function (small \( h' \)). A shallow uptake function indicates that the amount of Ca taken up by the SR is not very insensitive to the magnitude of cytosolic free [Ca] . For example, in the two cases shown in Fig. 6, when SERCA activity was normal, Ca uptake following the large release beat was much greater than that following the small release beat (Fig. 6A). However, when SERCA activity was reduced as in HF, the Ca taken up by the SR following the large beat was actually less than that of the small release beat (in this case \( h' < 0 \)) (Fig. 6B), which is the mechanism promoting alternans based on Eq. (2). Since this mechanism of alternans does not depend on refractoriness, alternans can occur at normal or slow heart rates, as indeed observed in experiments [29,33,35].

As shown in Fig. 2D, HF remodeling steepens the fractional SR release curve and TT disruption made it even steeper. Moreover, TT disruption results in a higher SR load (Fig. 2C). Therefore, in this mechanism of alternans, TT disruption promotes Ca alternans by steepening the fractional SR release curve and increasing SR load. However, as
Fig. 6. Sample recordings of $[\text{Ca}_i],[\text{Ca}]_{SR}, I_{\text{NCX}}, I_{\text{Ca,L}}$, and percentage of the RyR available for opening. A. From a simulation in Fig. 4. PCL = 500 ms, $[\text{Na}]_i = 11$ mM, and $p_{\text{LCC}} = 30\%$. B. From a simulation in Fig. 5. PCL = 1000 ms, $[\text{Na}]_i = 10$ mM, and $p_{\text{LCC}} = 30\%$. The amount of Ca uptake by SR for each of two alternating beats for the two conditions is marked.

Fig. 7. APD and Ca alternans in HF under free running conditions (current clamp). APD (A), peak $[\text{Ca}_i]$ (B), and SR load (C) versus PCL; and V (D), $[\text{Ca}_i]$ (E), and $[\text{Ca}_i]$ line scan (F) versus time. $[\text{Na}_i] = 10$ mM and $p_{\text{LCC}} = 30\%$. 

shown in Fig. 5A, alternans can occur without TT disruption, and TT disruption plays the role of a facilitator.

The second mechanism of Ca alternans is related to CRU refractoriness, and is also well-supported by experiments in which Ca alternans occurs without concomitant SR load alternans [35,36,39,40]. Theoretical analysis (called 3R theory) [37,41] shows that refractoriness alone is not sufficient, and the ability of CRUs to activate neighboring CRUs (Ca spark-induced secondary sparks) via diffusive coupling is also required. The mathematical description of this theory is

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

where \( N_{k+1} \) is the number of Ca sparks in the present beat, \( N_k \) is the number of sparks in the previous beat, \( N_0 \) is the total number of CRUs, and \( f \) is a nonlinear function describing the recruitment rate, which was derived as:

\[ f = 1 - \left[1 - \gamma(1 - \beta N_k/N_0)\right]^\gamma. \]

In Eqs. (3) and (4), \( \alpha \) is the probability or percentage of sparks triggered by the random opening of the LCCs (the first R of the 3R theory); \( \gamma \) is the probability that a spark recruits a neighboring CRU to fire (the second R); and \( \beta \) is the probability that a CRU which on the previous beat remains refractory during the present beat (the third R). \( M \) is the number of neighbors of a CRU. \( \alpha \) and \( \gamma \) are affected by many physiological factors (see Nivala and Qu [38]), such as LCC open probability, SR load, RyR leakiness, SERCA activity, NCX, and CRU spacing, and \( \beta \) is determined jointly by CRU refractoriness and PCL. Ca alternans occurs when \( \beta \) and \( \gamma \) are large, and \( \alpha \) is in the intermediate range (gray region in Fig. 8A). It is intuitively clear that in order to have an alternating beat-to-beat pattern, the refractory period of CRUs must be engaged. Otherwise the same number of CRUs would fire from beat to beat. However, due to the randomness of sparks, just engaging the refractory period is not sufficient to result in alternans. Coupling between CRUs is needed to locally synchronize the CRUs to fire so that more CRUs fire on one beat than on the alternate beat, resulting in alternans. However, if all the CRUs are triggered by their LCCs (high \( \alpha \)), there is no chance for recruitment to occur. Factors that decrease the number of sparks triggered by LCCs include: 1) reduced LCC conductance or open probability; 2) reduced LCC-RyR coupling fidelity (e.g., due to enlarged dyadic volume); and 3) TT disruption. In the first two cases, the beat-to-beat firing probability of a CRU is not 100% because of the reduced LCC conductance, open probability or reduced LCC-RyR coupling fidelity. That is, CRUs are not reliably triggered by their LCCs. The CRUs which are not activated by LCCs then provide the chance for spark-induced sparks to occur (this is why alternans requires \( \alpha < 1 \) in the 3R theory [37,41]).

In the third case, removal of LCC clusters provides the chance for spark-induced sparks to occur by creating orphaned RyR clusters. Thus, the role of TT disruption in promoting alternans is to allow recruitment to occur. As shown in Fig. 4A, TT disruption is required for alternans when LCC and LCC-RyR coupling are normal.

In summary (Fig. 8B), TT disruption results in orphaned RyR clusters, which reduces the number of CRUs activated by LCC clusters and thereby increases the opportunity for recruitment. In the SR Ca load-dependent mechanism of alternans, recruitment causes a steeper SR fractional release curve which promotes alternans. In the RyR refractoriness-dependent mechanism of alternans, the recruitment allows synchronization of sparks to promote alternans. TT disruption may increase SR load which is also a factor promoting Ca alternans.

4.2. Implications to Ca alternans in HF

Electrophysiological remodeling in HF is complex and evolves over time as HF progresses [15–18]. As shown in animal studies [26,48], the Ca transient can be increased in early stages of HF, but becomes prolonged with reduced amplitude in later stages of HF. It is likely that in the early stages, SERCA is still relatively normal and Ca is increased due to elevated [Na], while in the late stages, SERCA down-regulation and RyR leakiness depress the Ca transient. However, TT disruption may start early in the development of HF [6,49]. Based on these observations, coupled with the mechanistic insights from our computational results, one can speculate that different mechanisms may be involved in Ca alternans at different stages of HF. In the early stage when SERCA activity is still relatively normal, Ca elevation due to [Na], elevation increases both \( \alpha \) and \( \gamma \) with or without increased RyR leakiness (from the black star to the yellow star in Fig. 8A) [38]. However, this does not cause alternans. TT disruption then reduces \( \alpha \) to allow spark-induced sparks to occur, which results in alternans (from the yellow star to the red star in Fig. 8A). Therefore, TT disruption plays a very important role in promoting alternans in this stage. In the later stages of HF where SERCA is down-regulated, leaky RyR and TT disruption result in a steeper SR fractional release curve and shift the curve to a lower Ca load (Fig. 2D), promoting Ca alternans by the SR load-dependent mechanism. In this setting, TT disruption plays a less important role and increased RyR leakiness and SERCA down-regulation are the key factors. Note that in the early stage of HF, alternans requires the presence of refractoriness, and thus, tends to occur at fast heart rates, and may not be seen at normal heart rates. In the late stage of HF, however, refractoriness is not required, alternans can occur at either fast or slow heart rates, which may be responsible for pulsus alternans seen at normal heart rates in patients with severe end-stage HF.
4.3. Limitations

Several limitations should be mentioned. Although our cell model contains a spatially-distributed CRU network, the CRU model lacks asymmetrical details of geometry, RyR localization, and heterogeneous RyR cluster distribution [50–52]. In addition, in our simulations, the TTs were randomly disrupted, which may not be realistic. Spatial correlation or coarser patterns of TT disruption may cause more complex spatiotemporal behaviors of Ca alternans and other Ca cycling dynamics which need to be addressed in future studies. Refractoriness of Ca release is controversial, with time constants measured experimentally ranging from 100 to 650 ms [53–57]. Some studies have concluded that the refractoriness is mainly related to SR refilling and luminal Ca sensing, while others show that it is due to recovery of the RyR channels. The refractoriness in our model is caused by RyR recovery from inactivation and the time constant is in the slow range [Fig. 6]. This accounts for alternans occurring at PCL = 500 ms or less when alternans is refractoriness-dependent. A related issue is our use of the RyR model by Stern et al. [58], in which spark termination is caused by cytosolic refractoriness-dependent. A related issue is our use of the RyR model by Stern et al. [58], in which spark termination is caused by cytosolic refractoriness-dependent.

None.

Acknowledgements

This work is supported by grants from National Institute of Health P01 HL078931, R26 HL118041, American Heart Association, Western States Affiliate, Beginning Grant-in-Aid 14BGIAS470026 (M.N.), and the Laubisch and Kawada Endowments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jmcc.2014.10.018.

References

[1] Soeller C, Cannel MB. Examination of the transverse tubular system in living cardiac myocytes by 2-photon microscopy and digital image–processing techniques. Circ Res 1996;84:266–75.
[2] Brette F, Orchard C. Resurgence of cardiac T-tubule research. Physiology (Bethesda) 2007;22:167–73.
[3] Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ, et al. Loss of T-tubules and other changes to surface topology in ventricular myocytes from failing human and rat heart. Proc Natl Acad Sci U S A 2009;106:6854–9.
[4] Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. Proc Natl Acad Sci U S A 2006;103:4305–10.
[5] Liao J, Stone RA, Tosteson TD, Saccare TA, Laal MA, Sjostrom L, et al. T-tubule disorganization and reduced synthesis of Ca2+–release in murine cardiomyocytes following myocardial infarction. J Physiology 2006;574:519–33.
[6] Wei S, Gao A, Chen B, Kutschke W, Xie YP, Zimmerman K, et al. T-tubule remodeling during transition from hypertrophy to heart failure. Circ Res 2010;107:529–31.
[7] Swift F, Franzini-Armstrong C, Oyaheu L, Egerer AU, Anderson KB, Christensen GM, et al. Extreme sarcoplasmic reticulum volume loss and compensatory T-tubule remodeling after Sen2 knockout. Proc Natl Acad Sci U S A 2012;109:3997–4001.
[8] Heinzel FR, Bito V, Biesmans L, Wu M, Dietre E, van Wesum F, et al. Remodeling of T-tubules and reduced synthesis of Ca2+–release in myocytes from chronically ische- mic myocardium. Circ Res 2008;102:338–46.
[9] Sachse IB, Torres NS, Savio-Galimberti Aiba TA, Kars DA, Tomasselli GF, et al. Subcel- lular structures and function of myocytes impaired during heart failure are restored by cardiac resynchronization therapy. Circ Res 2012;110:588–97.
[10] Wagner E, Lauterbuch MA, Koth T, Westphal V, Wibermann GS, Steibenherz JH, et al. Stimulated emission depletion live-cell super-resolution imaging shows prolifera- tive remodeling of T-tubular membrane structures after myocardial infarction. Circ Res 2012;111:402–14.
[11] Hong T-T, Smyth JW, Chu KY, Vogan JM, Fong TS, Jensen BC, et al. BINI is reduced and CaV1.2 trafficking is impaired in human failing cardiomyocytes. Heart Rhythm 2012;9:812–20.
[12] Saconogi L, Ferrantini C, Lotti J, Coppini R, Yan P, Loew LM, et al. Action potential pro- pagation in transverse-axial tubular system is impaired in heart failure. Proc Natl Acad Sci U S A 2012;109:5815–9.
[13] Orchard CH, Bryant SM, James AF, Do T-tubules play a role in arrhythmogenesis in cardiac ventricular myocytes? J Physiology 2013;591:4141–7.
[14] Hong T, Yang H, Zhang S-S, Cho HC, Kalashnikova M, Sun B, et al. Cardiac BINI folds T-tubule membrane, controlling ion flux and limiting arrhythmia. Nat Med 2014;20: 624–32.
[15] Tomasselli GF, Marbán E. Electrophysiological remodeling in hypertrophy and heart failure. Cardiovasc Res 1999;42:270–83.
[16] O’Rourke B, Kass DA, Tomasselli GF, Kaab S, Tunin R, Marban E. Mechanisms of al- tered excitation-contraction coupling in canine tachycardia-induced heart failure. I: experimental studies. Circ Res 1999;86:562–70.
[17] Pogwizd SM, Schlothauser K, Li L, Yuan W, Bers DM. Arrhythmogenic and contrace- trial dysfunction in heart failure: role of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. Circ Res 2001;88: 1159–67.
[18] Nattel S, Maguy A, Le Bouter S, Shv Y-H. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. Physiological Rev 2007;87:425–56.
[19] Traube L. Ein fall von pulsus bignonius nebst bemerkungen tiber die lebershwellungen bei klappefehlern und uber acut oder ber lern-terisch. Berlin Klin Wschr 1872:189.
[20] Rosenbaum DS, T-tubules: a mechanism for arrhythmogenesis comes of age after 100 years. J Cardiovasc Electrophysiol 2001;12:207–9.
[21] Rosenbaum DS, Jackson LE, Smith JM, Garan H, Ruskin JN, Cohen BJ. Electrical abnormalities and vulnerability to ventricular arrhythmias. N Engl J Med 1994;330: 235–41.
[22] Baraveli M, Salerno-Urriate D, Guezzetti D,Rossi MM, Zoli L, Forzani T, et al. Predictive significance for sudden death of microvoltage-T wave alternans in New York Heart Association class II congestive heart failure patients: a prospective study. Int J Cardiol 2005;105:235–7.
[23] Salerno-Urriate JA, De Ferrari GM, Klersy C, Pedretti RF, Tritto M, Sallusti L, et al. Prognostic value of T-wave alternans in patients with heart failure due to nonischemic cardiomyopathy: results of the ALPHA Study. J Am Coll Cardiol 2007;50:1896–904.
[24] Tracy RL, Suyajj D, Wan X, Hocke GS, Said TH, Gittinger M, et al. Heart failure enhances susceptibility to arrhythmogenic cardiac alternans. Heart Rhythm 2009;6: 251–9.
[25] Wasserstrom JA, Sharma R, Kapur S, Kelly JE, Kadish AH, Balke CW, et al. Multiple de- fects in intracellular calcium cycling in whole failing rat heart. Circ Heart Fail 2009;2: 223–32.
[26] Kapur S, Asstrup GL, Sharma R, Kelly JE, Arora R, Zheng J, et al. Early development of intracellular calcium cycling defects in intact hearts of spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 2010;299:H1843–53.
[27] Asstrup GL, Gupta DK, Kelly JE, O’Toole MJ, Nalians A, Chairy N, et al. Inhibition of the late sodium current slows T-tubule diluption during the progression of hyper- tensive heart disease in the rat. Am J Physiol Heart Circ Physiol 2013;305:H1088–97.
[28] Shiferaw Y, Watanabe MA, Garfiniel A, Weiss JN, Karma A. Model of intracellular cal- cium cycling in ventricular myocytes. Biophys J 2003;85:3686–88.
[29] Diaz MT, O’Neill SC, Eisner DA. Sarcoplasmic reticulum calcium content fluctuation is the key to cardiac alternans. Circ Res 2004;94:650–6.
[30] Qu Z, Shiferaw Y, Weiss JN. Nonlinear dynamics of cardiac excitation-contraction coupling: an iterated map study. Phys Rev E 2007;75:011927.

Disclosure

None.
[31] Tao T, O’Neill SC, Diaz ME, Li YT, Eisner DA, Zhang H. Alternans of cardiac calcium cycling in a cluster of ryanodine receptors: a simulation study. Am J Physiol Heart Circ Physiol 2008;295:H596–609.

[32] Xie LH, Sato D, Garfinkel A, Qu Z, Weiss JN. Intracellular Ca alternans: coordinated regulation by sarco(endo)plasmic reticulum calcium uptake, release, and leak. Biophys J 2008;95:3100–10.

[33] Li Y, Diaz ME, Eisner DA, O’Neill S. The effects of membrane potential, SR Ca2+ content and RyR responsiveness on systolic Ca2+–alternans in rat ventricular myocytes. J Physiol 2009;587:1283–92.

[34] Huertas MA, Smith GD, Gyorke S. Ca2+ alternans in a cardiac myocyte model that uses moment equations to represent heterogeneous junctional SR Ca2+–. Biophys J 2010;99:377–87.

[35] Huser J, Wang YG, Sheehan KA, Lipusis SL, Blatter LA. Functional coupling between glycolysis and excitation-contraction coupling underlies alternans in cat heart cells. J Physiol 2000;524(Pt 3):791–806.

[36] Picht E, DeSantiago J, Blatter LA, Bers DM. Cardiac alternans do not rely on diastolic sarcoplasmic reticulum calcium content fluctuations. Circ Res 2006;99:740–8.

[37] Rovetti R, Cui X, Garfinkel A, Weiss JN, Qu Z. Spark-induced sparks as a mechanism of intracellular calcium alternans in cardiac myocytes. Circ Res 2010;106:1582–91.

[38] Nivala M, Qu Z. Calcium alternans in a coupled network model of ventricular myocytes: role of sarcoplasmic reticulum load. Am J Physiol Heart Circ Physiol 2012;303:H541–52.

[39] Shkryl VM, Maxwell JT, Domeier TL, Blatter LA. Refractoriness of sarcomplasmic reticulum Ca release determines Ca alternans in atrial myocytes. Am J Physiol Heart Circ Physiol 2012;302:H2310–20.

[40] Wang L, Myles RC, De Jesus NM, Ohlendorf AKP, Bers DM, Ripplinger CM. Optical mapping of sarcoplasmic reticulum Ca2+– in the intact heart: ryanodine receptor refractoriness during alternans and fibrillation. Circ Res 2014;114:1410–21.

[41] Qu Z, Nivala M, Weiss JN. Calcium alternans in cardiac myocytes: order from disorder? J Mol Cell Cardiol 2013;58:100–9.

[42] Nivala M, de Lange E, Rovetti R, Qu Z. Computational modeling and numerical methods for spatiotemporal calcium cycling in ventricular myocytes. Front Physiol 2012;3:114.

[43] Shannon TR, Wang F, Bers DM. Regulation of cardiac sarcoplasmic reticulum Ca2+ release by luminal [Ca2+] and altered gating assessed with a mathematical model. Biophys J 2005;89:4096–110.

[44] Bers DM. Calcium cycling and signaling in cardiac myocytes. Annu Rev Physiol 2008;70:23–49.

[45] Shannon TR, Pogwizd SM, Bers DM. Elevated sarcoplasmic reticulum Ca2+ leak in intact ventricular myocytes from rabbits in heart failure. Circ Res 2003;93:592–4.

[46] Qu Z, Garfinkel A, Chen PS, Weiss JN. Mechanisms of discordant alternans and induction of reentry in simulated cardiac tissue. Circulation 2000;102:1664–70.

[47] Escobar AL, Valdivia HH. Cardiac alternans and ventricular fibrillation: a Bad case of ryanodine receptors re overrun on their duty. Circ Res 2014;114:1369–71.

[48] Shorofsky SR, Aggarwal R, Corretti M, Raffa JM, Strum JM, Al-Seikhan BA, et al. Cellular mechanisms of altered contractility in the hypertrophied heart: big hearts, big sparks. Circ Res 1999;84:424–34.

[49] Shah SJ, Aistrup GL, Gupta DR, O’Toole MJ, Nahhas AF, Schuster D, et al. Ultrastructural and cellular basis for the development of abnormal myocardial mechanics during the transition from hypertension to heart failure. Am J Physiol Heart Circ Physiol 2014;306:H88-100.

[50] Hakel J, Edwards AG, Yu Z, Rekenes-Huskey PM, Michalova AP, Mccammon JA, et al. Modeling cardiac calcium sparks in a three-dimensional reconstruction of a calcium release unit. J Physiol 2012;590:4403–22.

[51] Cannell MB, Kong CH, Imtiaz MS, Laver DR. Control of sarcoplasmic reticulum Ca2+ release by stochastic RyR gating within a 3D model of the cardiac dyad and importance of induction decay for CICR termination. Biophys J 2013;104:2149–59.

[52] Baddeley D, Jayasinghe ID, Lam L, Rossberger S, Cannell MB, Soeller C. Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. Proc Natl Acad Sci U S A 2009;106:22275–80.

[53] Cheng H, Lederer MR, Lederer WJ, Cannell MB. Calcium sparks and [Ca2+]i waves in cardiac myocytes. Am J Physiol 1996;270:C148–59.

[54] Dell’Principe F, Egger M, Niggl E. Calcium signalling in cardiac muscle: refractoriness revealed by coherent activation. Nat Cell Biol 1999;1:323–9.

[55] Soblue EA, Song LS, Lederer WJ. Local recovery of Ca2+ – release in rat ventricular myocytes. J Physiol 2005;565:441–7.

[56] Belevych AE, Terentyev D, Terentyeva R, Ho HT, Garfinkel I, Bonilla JM, et al. Shortened Ca2+– signaling refractoriness underlies cellular arrhythmogenesis in a postinfarction model of sudden cardiac death. Circ Res 2012;110:569–77.

[57] Wasserstrom JA, Sharma R, O’Toole MJ, Zheng J, Kelly JE, Shryock J, et al. Ranolazine antagonizes the effects of increased late sodium current on intracellular calcium cycling in rat isolated intact heart. J Pharmacol Exp Ther 2009;331:382–91.

[58] Stern MD, Pizarro G, Ho HT, Gyorke I, Bonilla JM, Soeller C. Calcium–calmodulin signaling in cardiac myocytes: functional coupling between glycolysis and excitation-contraction coupling underlies alternans and fibrillation. Circ Res 2005;96:376–89.

[59] Shefranow Y, Sato D, Karma A. Coupled dynamics of voltage and calcium dy.

[60] Restrepo JC, Weiss JN, Karma A. Calcequinin-mediated mechanism for cellular calcium transient alternans. Biophys J 2008;95:7367–89.

[61] Shiferaw Y, Sato D, Karma A. Coupled dynamics of voltage and calcium in paced cardiac cells. Phys Rev E Stat Nonlin Soft Matter Phys 2005;71:021903.

[62] Groenendaal W, Ortega FA, Krogh-Madsen T, Christini DJ. Voltage and calcium dynamics both underlie cellular alternans in cardiac myocytes. Biophys J 2014;106:2222–32.