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Supplemental Information

Mechanism for Triggered Waves in Atrial Myocytes

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Supplemental Material

I. Diffusion Parameters

Parameters of the Restrepo-Karma computational cell model. All model parameters not listed bellow are the same as in the original model [1, 2].

Table 1. Diffusion time constants linking internal sites.

Parameter	Description	Value
τ_i^T	Transverse cytosolic diffusion time	$1.47ms$
τ_i^L	Longitudinal cytosolic diffusion time	$1.16ms$
τ_s^T	Transverse submembrane diffusion time	$0.71ms$
τ_s^L	Longitudinal submembrane diffusion time	$0.85ms$
τ_{NSR}^T	Transverse NSR diffusion time	$3.60ms$
τ_{NSR}^L	Longitudinal NSR diffusion time	$12.0ms$

Table 2. Diffusion time constants linking internal and peripheral sites.

Parameter	Description	Value
τ_i^T	Transverse cytosolic diffusion time	$2.93ms$
τ_i^L	Longitudinal cytosolic diffusion time	$2.32ms$
τ_s^T	Transverse submembrane diffusion time	$1.42ms$
τ_s^L	Longitudinal submembrane diffusion time	$1.7ms$
τ_{NSR}^T	Transverse NSR diffusion time	$7.2ms$
τ_{NSR}^L	Longitudinal NSR diffusion time	$24.0ms$

Table 3. RyR parameters.

Parameter	Description	Value
K_u	CSQN-unbound opening rate	$1.4 \times 10^{-4}(\mu M)^{-2}ms^{-1}$
N	Number of channels in RyR cluster	100
γ	Exponent of Ca binding	2.5

II. Robustness of triggered waves to changes in model parameters

In this section we analyze the robustness of our main results to model parameter changes. Our general approach is to compute the mean waiting time to Ca waves with boundary excitations (T_{tr}) and without boundary excitations (T_{sp}). These quantities are computed across a range of model parameters, and the results are compared to that shown in Figure 6 of the manuscript.

1. Diffusion. Since the diffusion coefficient of Ca in the intracellular space is not known with precision, we will also consider the case where the effective diffusion coefficient linking submembrane and cytosolic sites is decreased by a factor of 2. In this case the time scales $\tau_i^L, \tau_i^T, \tau_s^L, \tau_s^T$ given in Table 1 and 2 are increased by a factor of 2. For this set of parameters the velocity of a longitudinal wave is in the range $50 - 100 \mu m/s$ for SR loads in the range $1330 - 1500 \mu M$. In Figure s1A we plot the waiting time to a triggered (T_{tr}) and spontaneous (T_{sp}) wave for this set of model parameters. Here, the cell is paced with the same pacing protocol as that used to compute Figure 6. Since diffusion is lowered the onset of wave propagation in this model increases to $1330 \mu M$ compared to $1200 \mu M$ in the previous model. Our results show that the timing of triggered and spontaneous Ca waves is qualitatively the same as in our previous model. Namely, that triggered waves occur with shorter latency than spontaneous waves for a broad range of SR loads. Hence, with this parameter set the timing to Ca waves is qualitatively similar as that found using the parameters in Table 1 and 2. As in the previous case we note that when the SR load is decreased the waiting time to Ca waves rises rapidly in which $T_{tr} > T_{sp}$. This is because the AP induced Ca release leads to a partial depletion of the SR which increases the waiting time to a spontaneous Ca wave. However, in this case the timing to waves is substantially longer than the AP so that Ca waves in this regime of SR loads are not observed during pacing.

2. The proximal and submembrane volume of non-junctional sites. In this section we consider variations in the internal volumes of our simplified compartmental model. As a starting point we will first consider variations in the proximal volume v_p. Our computational model reveals that the onset of Ca waves is extremely sensitive to changes in v_p. This is because v_p dictates the rate of change of the proximal Ca concentration c_p which regulates RyR kinetics. In our studies we found that increasing v_p by 50% essentially abolishes Ca waves for SR loads bellow $1500 \mu M$. Thus, we found it necessary to increase the RyR current conductance by 10%. When this parameter change is made then Ca waves readily occur for SR loads in the range $1200 - 1500 \mu M$. For this set of parameters we plot T_{tr} and T_{sp} in Figure s1B. In this case we find that $T_{tr} < T_{sp}$ for a broad range of SR loads. In effect, both the waiting time to triggered waves and to spontaneous waves increased proportionally. Thus, under these model parameter changes we find that triggered waves occur with shorter latency than spontaneous waves. Similarly, in Figure s1C we plot the waiting times for the case where the submembrane volume v_s is increased by

50%. Here, we find that triggered waves again occur with shorter latency. These simulations indicate that an increase in the internal volume parameters prolong both triggered and spontaneous waves in a proportional manner. Therefore, the qualitative aspects of our main results are robust to changes in the internal volume parameters.

3. Action potential shape. We have also considered the case where the system is paced with a triangular AP. This AP shape is more similar to the AP of atrial myocytes. In this case we find that the distribution of subcellular Ca release in response to the AP is qualitatively similar to that of the AP used in the manuscript. In Figure s2 we repeat the simulation of Figure 4, using a triangular AP (red line). Under these conditions we find that the distribution of subcellular Ca is similar to the case considered in the manuscript. In both cases we find that triggered waves occur during the AP followed by spontaneous waves which have a much longer latency. Hence, the main results presented here should apply for a broad range of AP shapes. In future work it will be necessary to investigate the system behavior in the case where the voltage is unclamped.

II. Subcellular Ca during rapid pacing

In Figure s3 we show the spatial distribution of subcellular Ca during rapid pacing at $170ms$. In this simulation the cell is driven for 40 beats. Here, we observe that after many beats triggered waves begin to propagate into the cell interior generating an aperiodic response in the whole cell Ca transient c_i. In this case we see clearly that triggered Ca waves propagate across paced beats similarly to that seen in our experimental linescan images (Figure 2B-C).

III. β-adrenergic stimulation

In this section we investigate the effect of β-adrenergic stimulation on the beat-to-beat response of the cell. In Figure s4 we plot the last 16 beats for a simulation in which the cell was paced for 40 beats at $CL = 250ms$. In Figure s4A we show the Ca transient (black line), and in Figure s4B we show the subcellular Ca along the linescan position (b). Here, we see that triggered waves occur at steady state and the Ca transient exhibits an aperiodic response. To model the effect of isoproterenol we then increase the LCC current conductance by 15%, and also increase the SERCA conductance by a factor of 2. In Figure s4A (red line) we show the Ca transient at steady state, and in Figure s4C we show the corresponding linescan. Under these parameter settings we find that the Ca transient and the subcellular Ca release is effectively periodic in time. This result is consistent with Figure 2E where we find that isoproterenol leads to a periodic beat-to-beat response. In effect the increased SERCA activity and LCC conductance leads to enhanced Ca loading in the SR along with a stronger signaling fidelity. These changes lead to the nucleation of multiple triggered waves at each beat. When this occurs the beat-to-beat variation of Ca release is substantially reduced and Ca release becomes synchronous. Indeed, in Figure 2E we see that during the pause multiple spontaneous Ca waves occurred, which indicates that the SR load was substantially

elevated under these conditions. Here, we point out that the increase in SERCA conductance ensures that the SR is replenished by the end of each beat, which is crucial to ensure a stable beat-to-beat response. For normal SERCA conductance the SR load does not fully recover after a full release, so that the beat-to-beat response becomes aperiodic. This result suggests that β-adrenergic stimulation effectively synchronizes Ca release by promoting multiple triggered Ca waves in the cell, and allowing full recovery to ensure a similar release on the next beat.

Captions

Figure s1. Plot of the mean waiting time to a Ca wave. Red line (T_{tr}) is corresponds to the waiting time in the case where the cell is driven by an AP with triggered Ca release at the cell boundary. Black line (T_{sp}) corresponds to the waiting time to spontaneous Ca waves. In this case diffusion between junctional and non-junctional sites is set to zero. Model parameters used are: (A) Diffusion time scales linking submembrane and cytosolic volumes are increased by a factor of 2. (B) The proximal space volume v_p is increased by 50%. (C) The submembrane volume v_s is increased by 50%.

Figure s2. (A) Ca transient and AP clamp during pacing for one beat with an initial SR load of $1230\mu M$. (B) Simultaneous linescans at position (b) and the spatial averaged linescan (av).

Figure s3. Spatially distributed cell model under rapid pacing. In this simulation the cell is paced for 40 beats at $CL = 170ms$. (A) Ca transient (c_i) vs time (black line). Red line is the voltage clamp driving the system. (B) Line scan at position (b) through the cell center. (C) Spatially averaged line scan (av).

Figure s4. Effect of isoproterenol on the beat-to-beat dynamics. In this simulations isoproterenol is simulated by increasing LCC conductance by 15% and SERCA by a factor of 2. (A) Ca transient c_i for normal (black line) and under simulated isoproterenol conditions (red line). (B) Linescan through position (b) showing triggered waves under normal conditions. (C) Linescan through position (b) under conditions of isoproterenol.

References

1. Restrepo JG, Weiss JN and Karma A. Calsequestrin-mediated mechanism for cellular calcium transient alternans. *Biophysical journal*. 2008;95:3767-89.
2. Restrepo JG and Karma A. Spatiotemporal intracellular calcium dynamics during cardiac alternans. *Chaos*. 2009;19:037115.