EXPLORING SR CALCIUM AND CYTOSOLIC CALCIUM WAVE DYNAMICS USING A 3D STOCHASTIC MYOCYTE MODEL

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INTRODUCTION

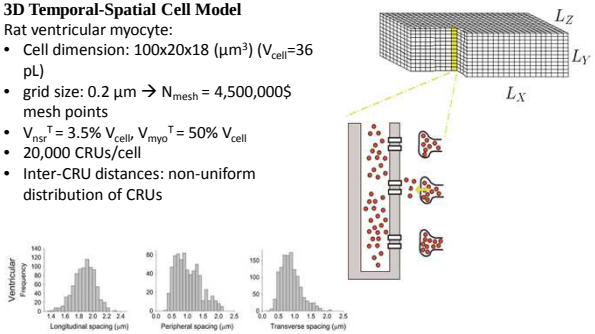
The local regulation of intracellular calcium has been widely known to play an important role in the normal excitation-contraction coupling in the cardiac myocytes. While Ca^{2+} sparks do not normally trigger regenerative SR Ca^{2+} release (i.e. Ca^{2+} waves), under calcium overload conditions calcium sparks can trigger spontaneous calcium waves. While experimental imaging using confocal microscopy with fluorescent dyes is the major technique used to study calcium dynamics inside a cardiac myocyte, the dynamics of SR calcium during calcium overload is poorly understood and controversial. In the study presented here, we present a new temporal-spatial model of calcium sparks to examine this issue. The resolution is sufficiently high (100nm) so that all critical local fluxes can be properly considered. The model also takes into account the different expression levels of the sodium-calcium exchanger (NCX) and SERCA pumps near the SR Ca^{2+} release sites. From that, we want to demonstrate different factors contributing to the characteristics of Ca^{2+} sparks. This work includes an examination of the extent of SR calcium elevation, and how much calcium must be released to support the triggering of a sustained propagating Ca^{2+} wave within the cell. The findings presented here will be compared to published work in other studies.

3D WHOLE-CELL RAT MODEL

3D Temporal-Spatial Cell Model

Rat ventricular myocyte:

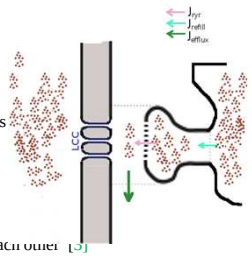
- Cell dimension: 100x20x18 (μm^3) (V_{cell}=36 pL)
- grid size: 0.2 µm → N_{mesh} = 4,500,000$ mesh points
- V_{nsr}^T = 3.5% V_{cell}, V_{myo}^T = 50% V_{cell}
- 20,000 CRUs/cell
- Inter-CRU distances: non-uniform distribution of CRUs



CRU MODEL

Each CRU:

- A dyad subspace: 300x300x12 (nm^3)
- A junctional SR: 300x300x50 (nm^3)
- A network SR:
- Cytoplasm
- Spark simulation: 36 RyR2 + 2 satellite clusters of RyR, each with 15RyR at 100nm distance
- Ca^{2+} wave simulation: 49 RyR2



Ryanodine receptor-2 (RyR2) [2]

- 2-state minimal model
- Stochastic gating, with allosteric coupling to each other [3]
- Both cytosolic Ca^{2+} sensitivity and luminal Ca^{2+} regulation.

Fluxes

- J_{efflux} : from subspace
- J_{refill} : from local NSR
- J_{ryr} : from JSR to subspace via opening RyR2s

CALCIUM WAVES - EXPERIMENT

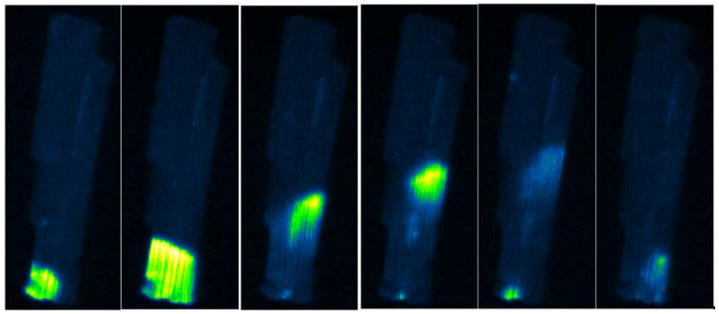


Fig. 1: Calcium waves under $[Ca]_o$ = 5mM overload condition, the repetitive waves occur at a particular sites for each cell. This suggest there is a more density of release sites surrounding that region that allows mass calcium release is high enough to trigger the wave. Some waves can sustain to the next end, while some decay and stop in between, which suggests a stochastic nature of the waves.

CALCIUM WAVES - SIMULATION

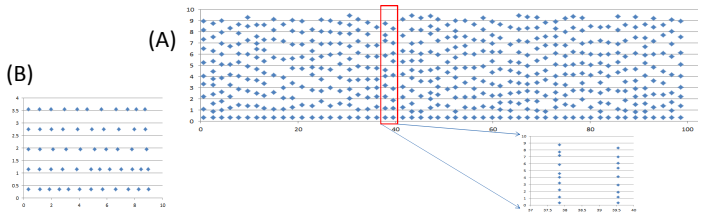


Fig. 2: The placement of calcium release sites **(A)** at one z-depth, **(B)** at one Z-disc. The distribution of inter-CRU distance is derived based on the experimental data.

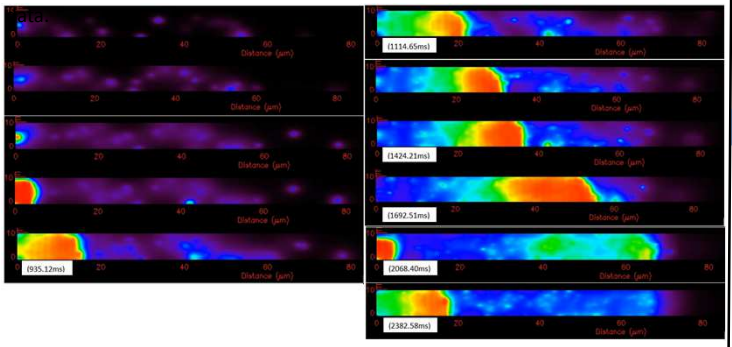


Fig. 3 Calcium overload ($[Ca]_{nsr}$=1.7mM, $[Ca]_i$=0.15µM), this computational model of the rat ventricular myocyte can reproduce a repetitive sustained calcium wave which typically initiates at one end of the cell. The initiation site typically occurs where release sites are closer together or at a boundary.

CALCIUM SPARKS

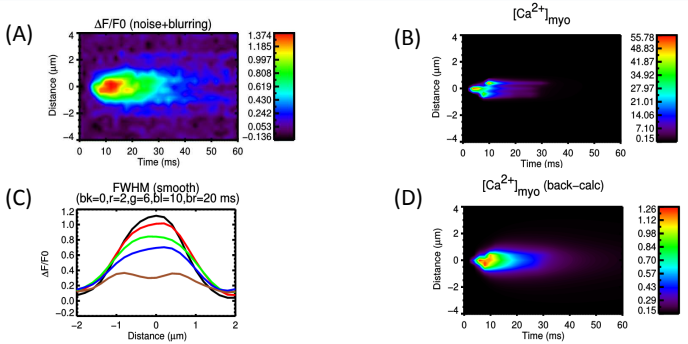


Fig. 4: (A) A simulated calcium sparks, **(B)** Free calcium shows the underlying structure of release site (the delayed activation of the two satellite clusters are invisible under fluorescence profile, **(C)** The profile of a calcium spark giving FWHM=1.85um (each color represents the snapshot at different time point after the peak (e.g. bk=0 means black line at 0ms delayed); **(D)** The free calcium profile using back-calculation method agrees with experimental estimates, however, it underestimates the real free myoplasmic calcium amplitude.

DYNAMICS OF SR CALCIUM DEPLETION WAVES

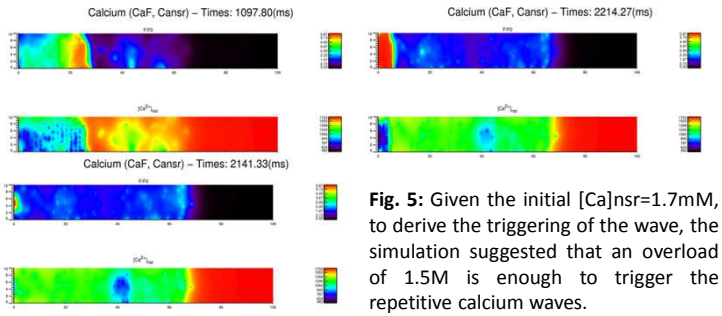


Fig. 5: Given the initial [Ca]nsr=1.7mM, to derive the triggering of the wave, the simulation suggested that an overload of 1.5M is enough to trigger the repetitive calcium waves.

CONCLUSIONS

The spatio-temporal model with realistic CRU distribution, CRU geometry suggests the following:

- The spark model can reproduce realistic spark characteristics (FWHM=1.85µm, duration, rise time) by using realistic RyR dynamics, buffering, geometry, and diffusion.
- The back-calculation method underestimate s the true free calcium.
- The non-uniform placement of CRU is important for calcium wave initiation and propagation. Under calcium overload.

ACKNOWLEDGMENTS

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