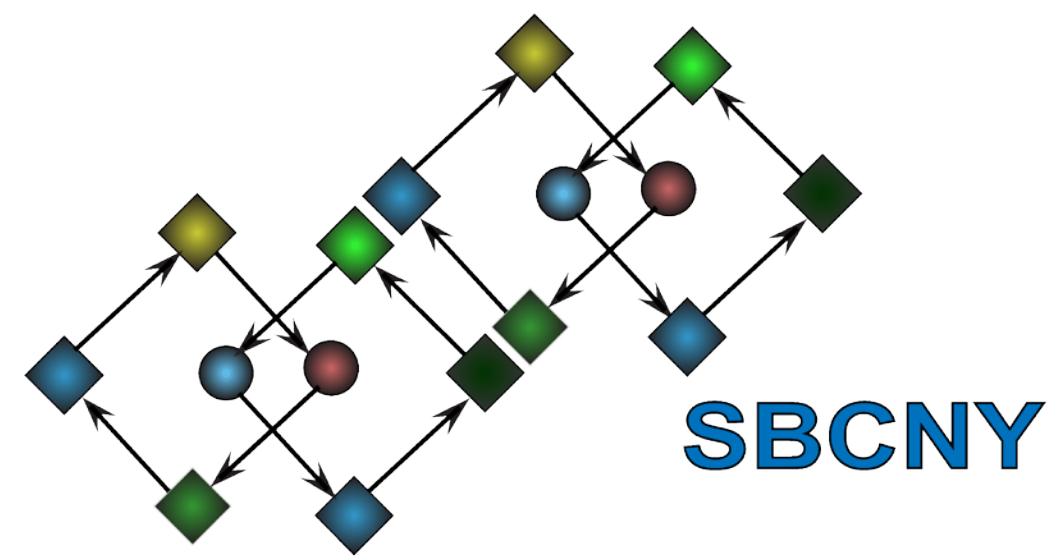




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Calcium Leakage from SR Ryanodine Receptors

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Abstract

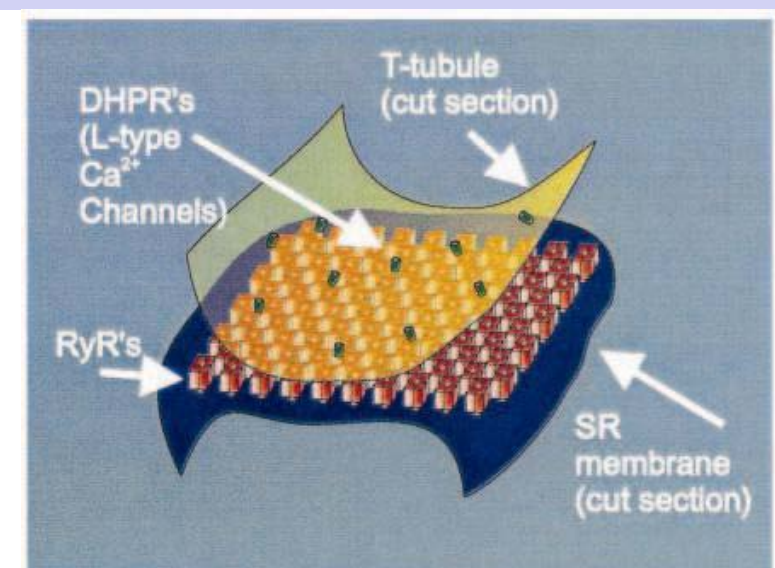
In ventricular myocytes, ryanodine receptors (RyR2s) are the intracellular ion channels responsible for release of Ca^{2+} from the sarcoplasmic reticulum (SR). During each cardiac cycle, many RyR2s are triggered by Ca^{2+} influx through L-type Ca^{2+} channels, resulting in a large increase in intracellular $[Ca^{2+}]$, which then leads to myocyte contraction. In resting cells, RyR2s can open spontaneously, resulting in "leak" of Ca^{2+} from the SR into the myoplasm. Because inappropriate leak of Ca^{2+} is thought to be linked to arrhythmias in disease states, it is important to obtain a quantitative understanding of SR Ca^{2+} leak.

A RyR2 opening spontaneously can open and release Ca^{2+} from the sarcoplasmic reticulum (SR). The Ca^{2+} released from the SR can trigger adjacent RyR2 to open through a positive feedback process known as Ca^{2+} -induced- Ca^{2+} -release. When many of the RyR2 within a cluster are opened, the release can be visualized experimentally as a Ca^{2+} spark. However, when only one or a few RyR2s open, the Ca^{2+} leakage that results cannot be observed directly. We used computational modeling to generate quantitative predictions of how different factors influence visible and invisible SR Ca^{2+} leak.

A modified Monte Carlo computational model of a RyR2 cluster, based on Sobie et al (2002) discoveries, was used to simulate the opening of the receptors and Ca^{2+} release from the SR. Understanding that the gating of RyR2 depends on the $[Ca^{2+}]$ in both the SR and the myoplasm, we varied the $[Ca^{2+}]$ in both these regions to observe the effects on each would have on RyR2 behavior. Using the data generated from the RyR2 model simulations, the Ca^{2+} leak was calculated. The volume of the region between the RyR2 clusters and the cell membrane was also varied because experiments suggest that the close spatial coupling between the SR and cell membranes may be disrupted in heart failure.

Our simulation results reveal that: 1) the probability of a spontaneous RyR2 opening will trigger a spark is dependent on the $[Ca^{2+}]$ in the SR, as $[Ca^{2+}]$ increased the probability increased. The $[Ca^{2+}]$ in the resting myoplasm made negligible differences. 2) The $[Ca^{2+}]$ in the myoplasm, however, have a greater effect on the rate of RyR2 opening than the SR $[Ca^{2+}]$. Increase in myoplasmic $[Ca^{2+}]$ increased the opening rate. 3) As a result, leak depends on the $[Ca^{2+}]$ in both region, but more in the myoplasm. 4) The leak was also dependent on the subspace volume. Increasing the volume leads to a greater invisible leak.

Background

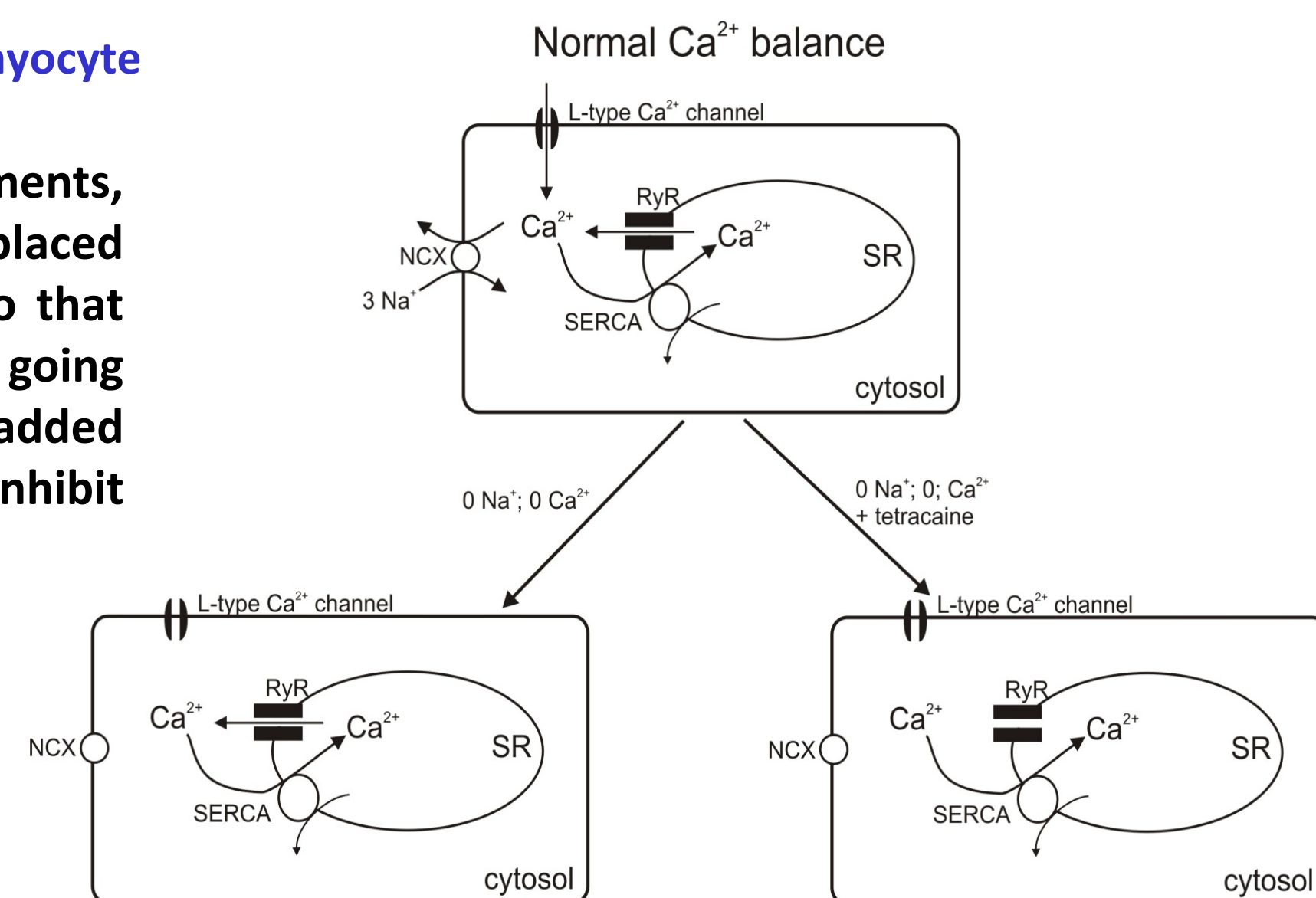


RyR2s between the T tubule and the SR are arranged in clusters consisting of roughly 100 channels.

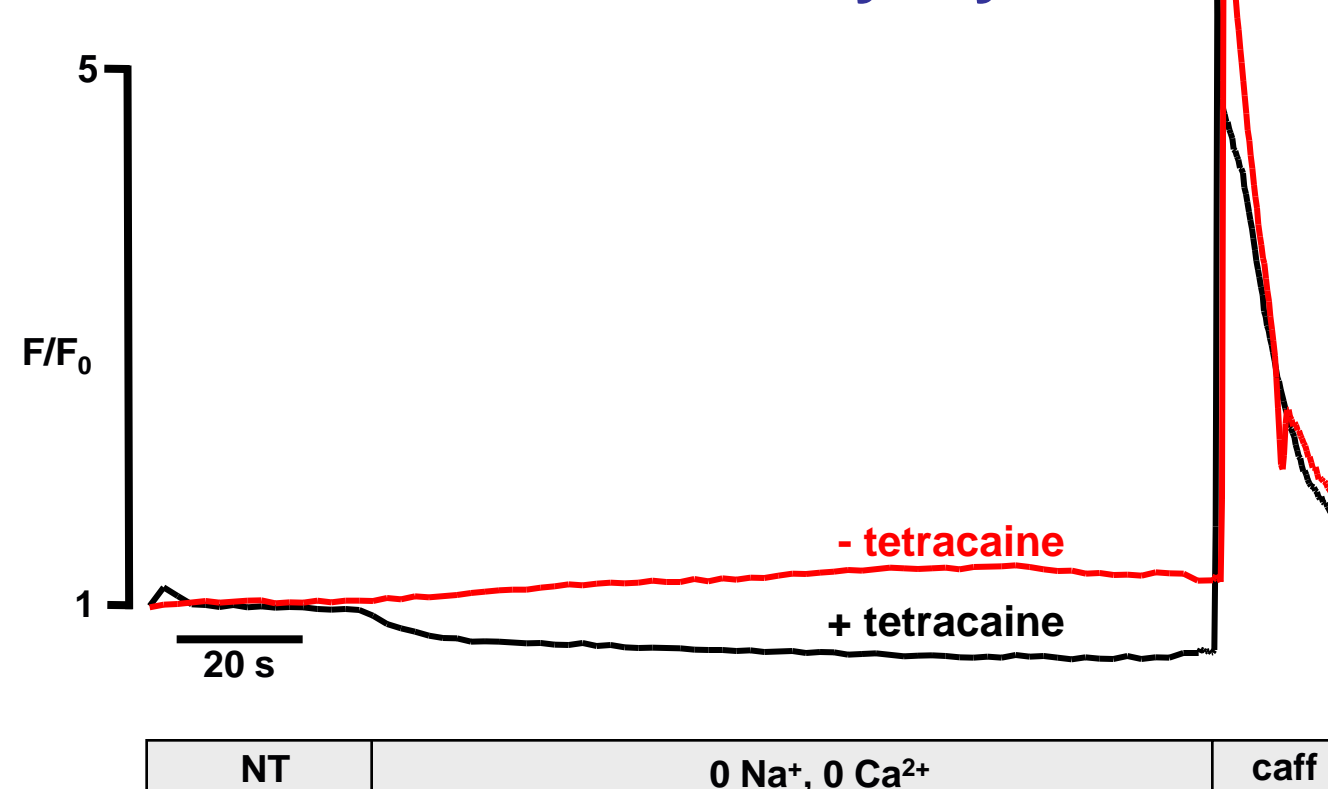
SR Ca^{2+} release through RyR2s contributes to both the normal Ca^{2+} transient in electrically stimulated cells and "leak" in resting myocytes.

SR Ca^{2+} leak in rat ventricular myocyte

In controlled experiments, ventricular myocytes are placed in solutions with no Ca^{2+} so that there is no external Ca^{2+} going into the cells. Tetracaine is added to one of the solutions to inhibit the opening of the RyR2s.



SR Ca^{2+} leak in rat myocyte



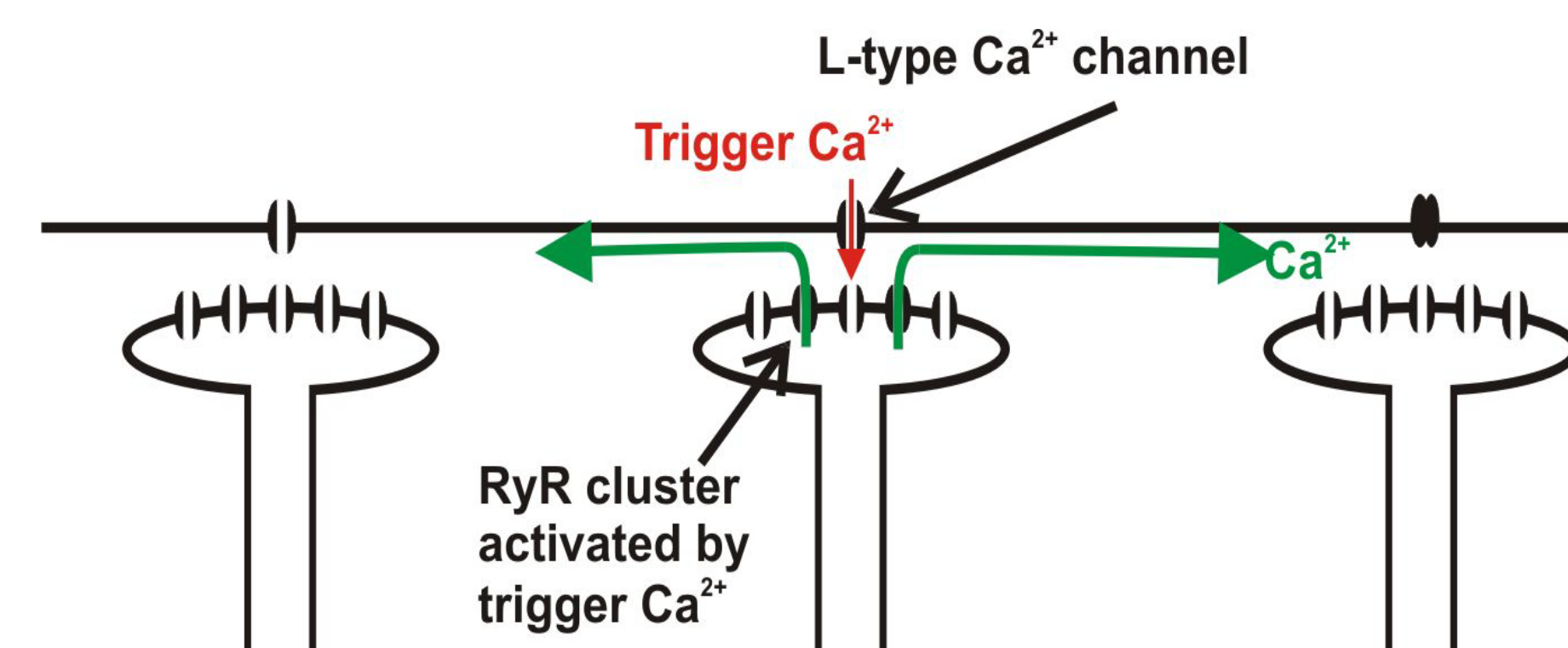
When the receptor is blocked by tetracaine, there is a decrease in fluorescence which indicates less Ca^{2+} is leaking out to the myoplasm. Therefore Ca^{2+} leaks from the SR via RyR2s when RyR2s are not blocked.

Objective

To use stochastic computational modeling of Ca^{2+} sparks to generate quantitative predictions of visible and invisible diastolic SR Ca^{2+} leak in ventricular myocytes. Specifically, we sought to determine the effects of:

- 1) changes in myoplasmic $[Ca^{2+}]$
- 2) changes in SR $[Ca^{2+}]$
- 3) changes in the volume of the dyadic space

Ca^{2+} Spark Model



Ca^{2+} released from a cluster of RyR2s can be visualized experimentally as a Ca^{2+} spark. The termination of a spark depends on depletion of $[Ca^{2+}]$ on the luminal side of the RyR2 and coupled gating between the RyR2s in a cluster. Ca^{2+} sparks in this model (Sobie et al., *Biophysical Journal* 83:59-78, 2002) terminate robustly.

Computational Details

Initial conditions:

sub-space and JSR $[Ca^{2+}]$ begin at the fixed values of myoplasmic and NSR $[Ca^{2+}]$. at time $t=0$, a single RyR2 opens.

Each stochastic trial simulated 59 ms.

Flux was calculated as $J = \int I_{RyR} \times k_{open} \times P_{trigger}$.

50 RyR2s present in each cluster

$$D_{RyR} = 4 \times 10^{-13} \text{ (}\mu\text{L ms}^{-1}\text{)}$$

$$J_{RyR} = N_{open} \times D_{RyR} \times ([Ca^{2+}]_{JSR} - [Ca^{2+}]_{ds}) / V_{ds}$$

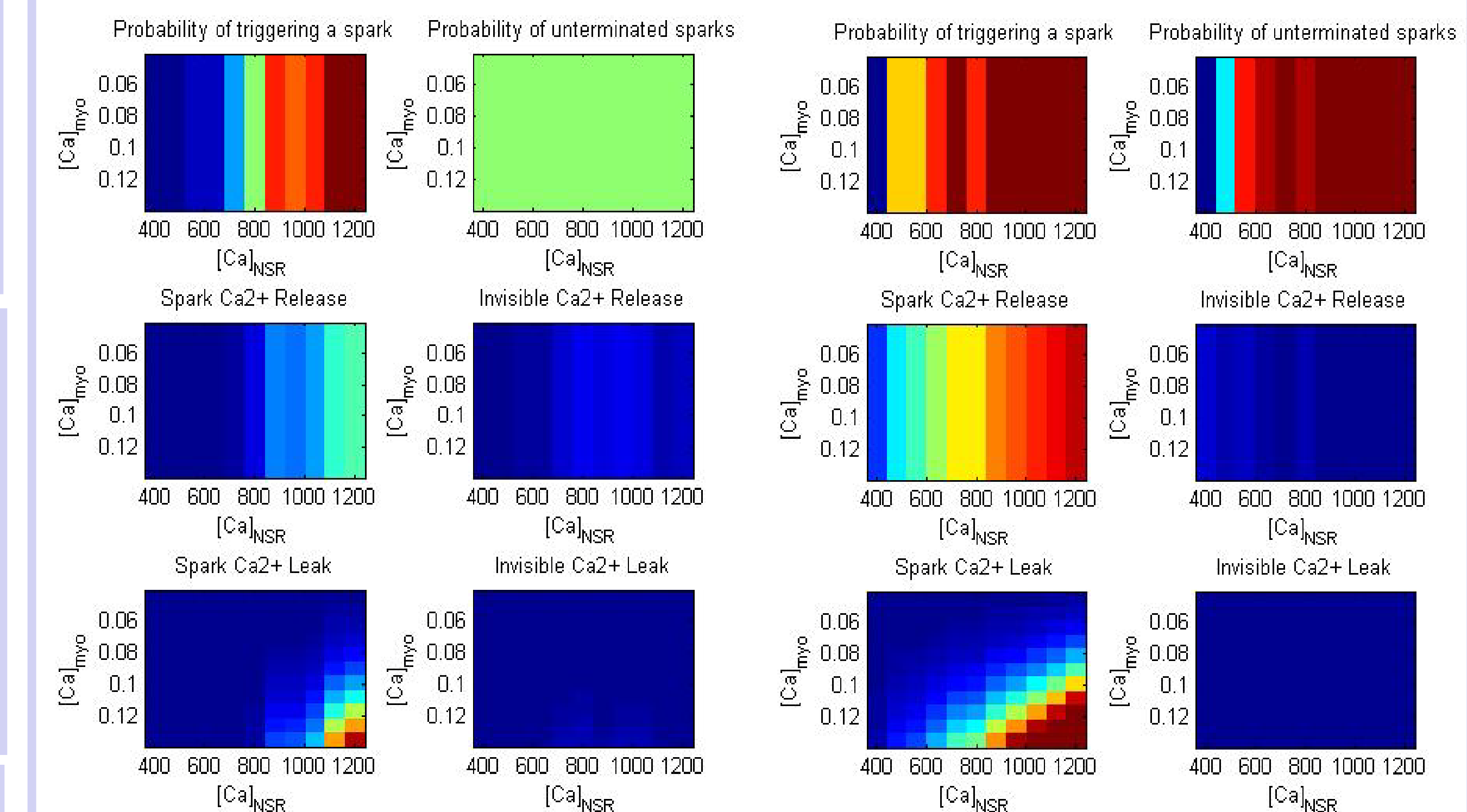
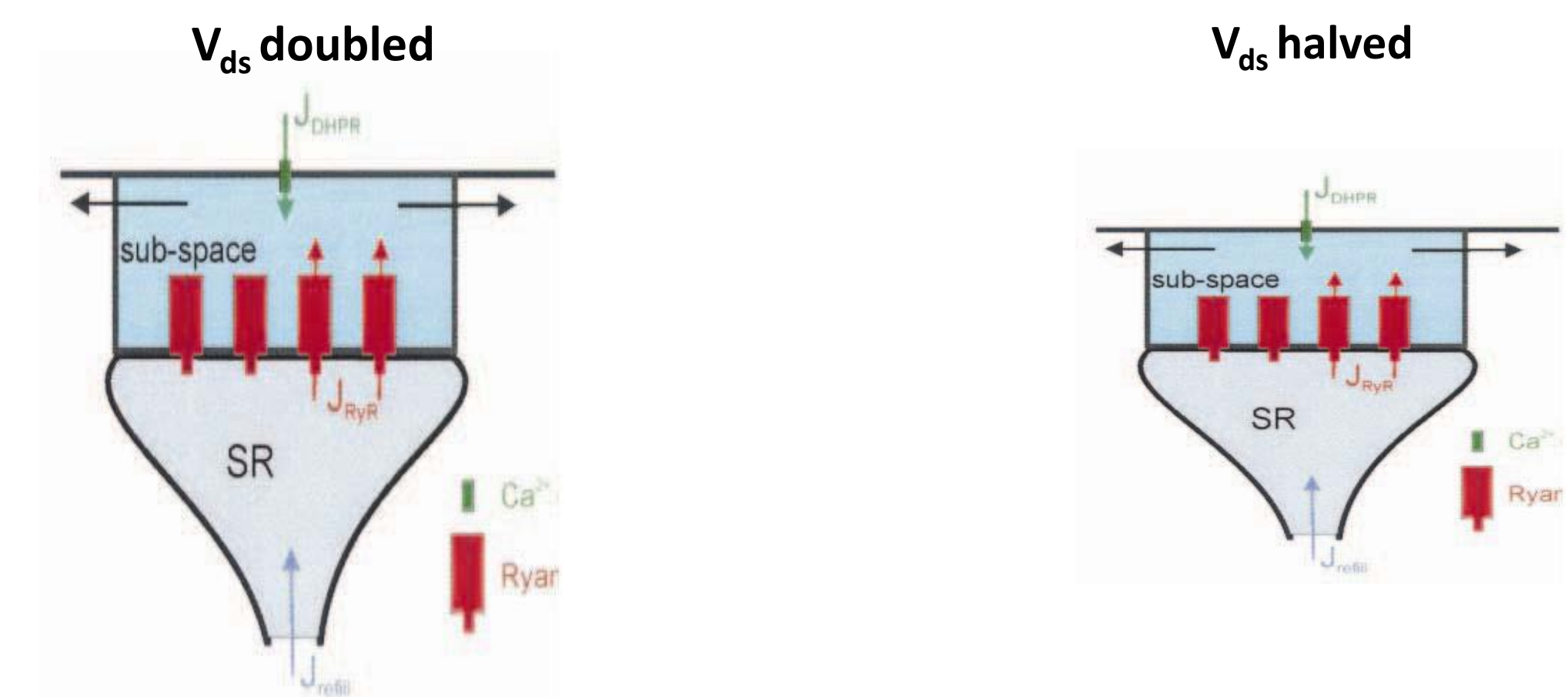
$$I_{RyR} = 1 \times 10^6 \times J_{RyR} \times 2F \times V_{ds}$$

$$K_m = 17.14 - 0.00686 [Ca^{2+}]_{JSR} \text{ (}\mu\text{M)}$$

$$k_{open} = 30 \cdot CF_{open} [Ca^{2+}]_{ds}^4 / ([Ca^{2+}]_{ds}^4 + K_m^4) \text{ (ms}^{-1}\text{)}$$

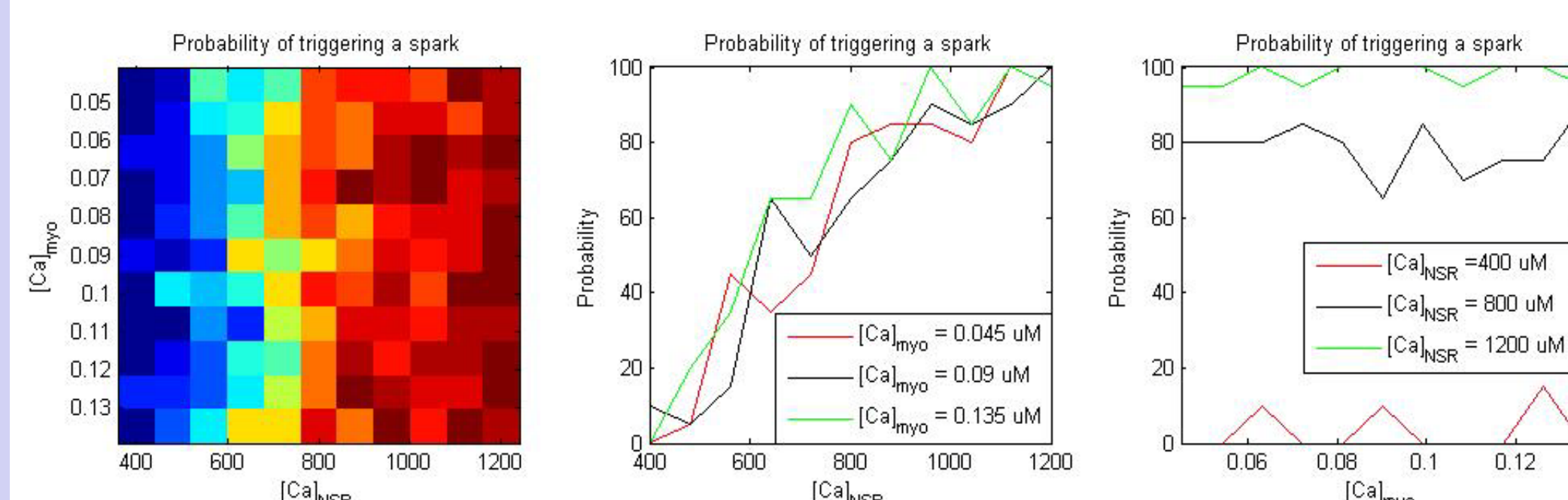
Results

In disease states, there can be structural changes in the T tubule which cause a greater gap between the sarcolemma and SR membrane or even orphan the RyR2s. The volume of the subspace (V_{ds}) in the model was varied to understand how this affects SR Ca^{2+} leak.

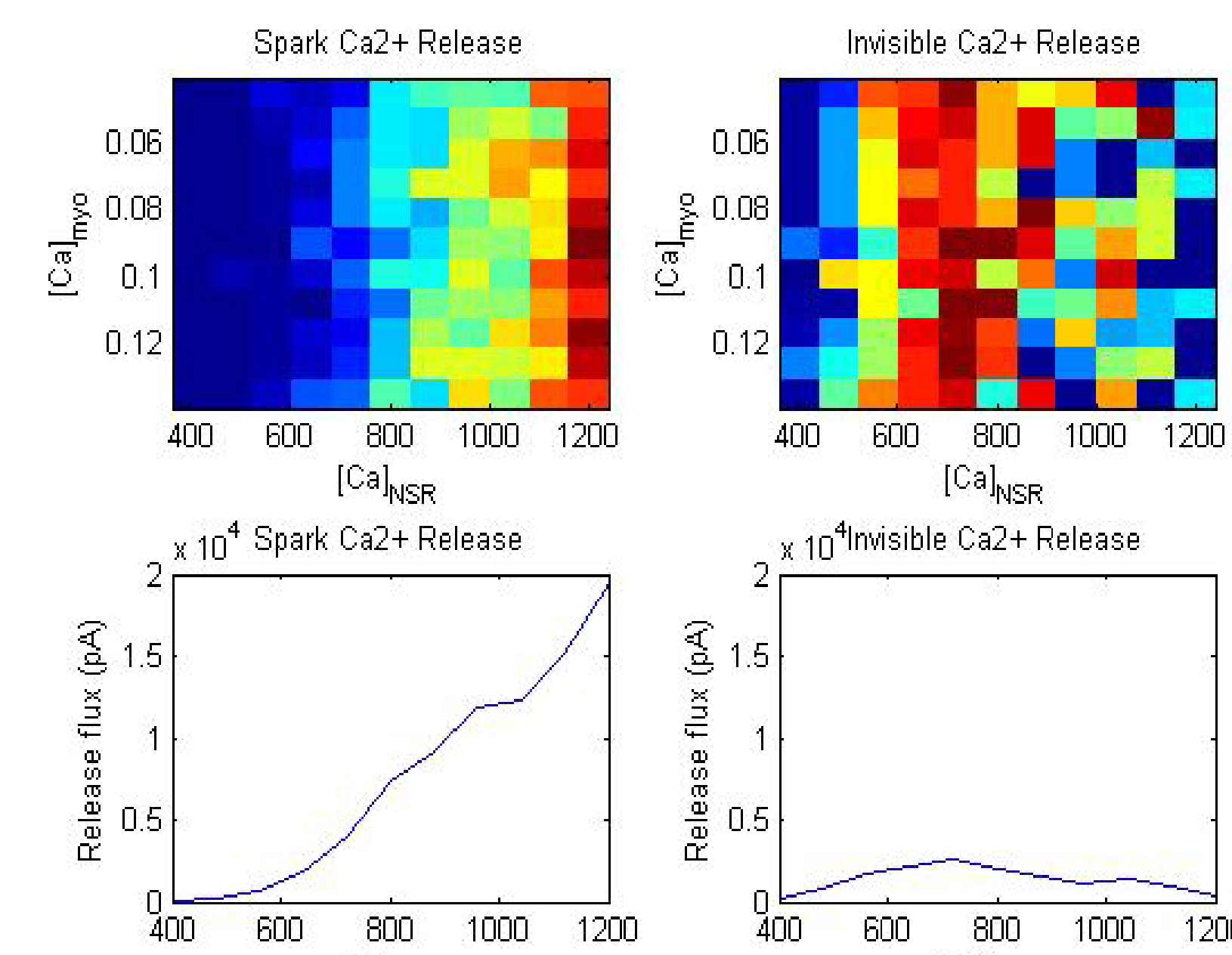


	V_{ds} doubled	V_{ds} halved
Untriggered sparks	No	Yes
Ca^{2+} release - spark	Decreased	Increased
Ca^{2+} release - leak	Increased	Decreased
Ca^{2+} release flux - spark	Decreased	Increased
Ca^{2+} release flux - leak	Increased	Decreased

Results



As the $[Ca^{2+}]$ in the NSR increases, the probability of triggering a Ca^{2+} spark increases. Changing the $[Ca^{2+}]$ in the myoplasm shows little effect on probability of triggering a spark.



When there is a spark, the amount of Ca^{2+} released is greater when the $[Ca^{2+}]$ is greater.

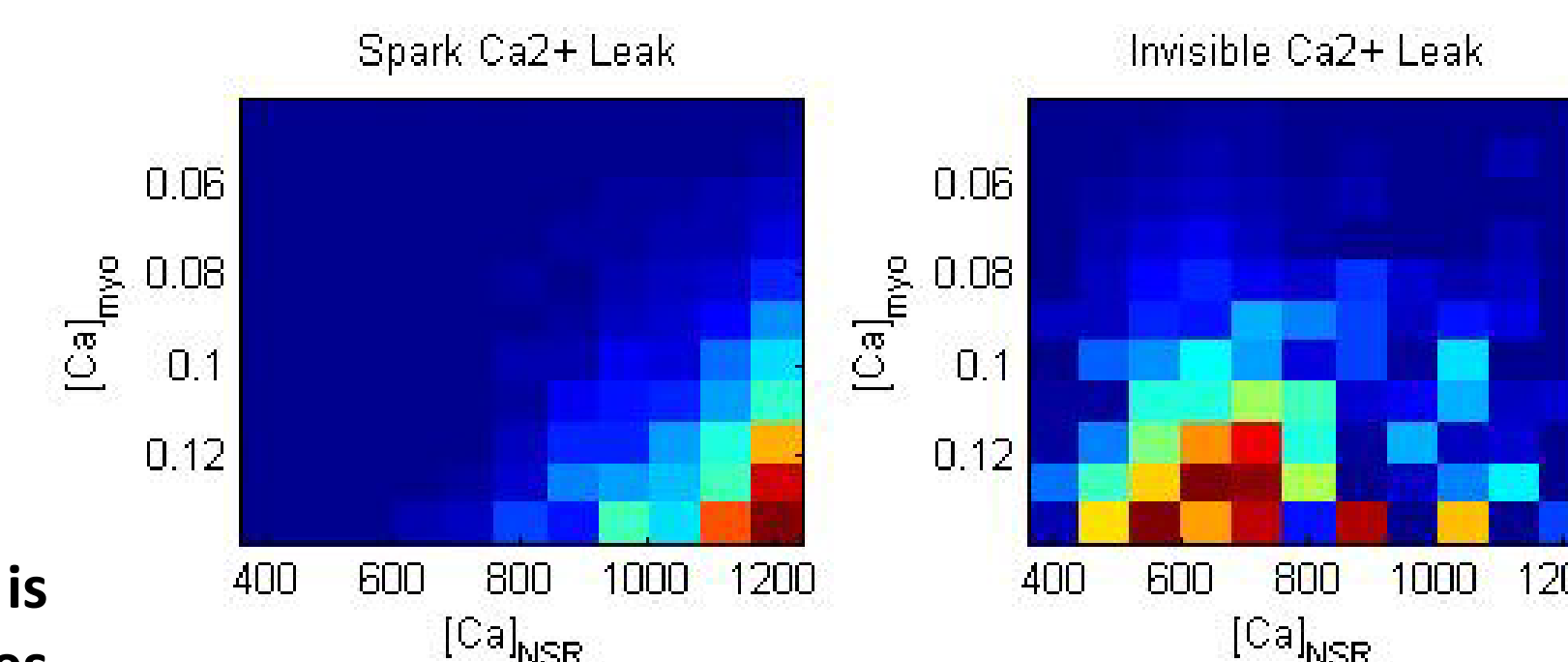
When there is no spark, there is also Ca^{2+} released, but it does not increase with the increase of NSR $[Ca^{2+}]$.

The flux, J , of Ca^{2+} release is calculated by multiplying three factors:

- 1) the probability of trigger a spark
- 2) the total amount of Ca^{2+} released during a typical spark
- 3) the opening rate of the RyR2, k_{open}

k_{open} is a function of the $[Ca^{2+}]$ in both the NSR and the myoplasm.

During a Ca^{2+} spark, the flux of Ca^{2+} release is increased as the $[Ca^{2+}]$ in both the NSR and the myoplasm are increased. When there is no spark, the flux of Ca^{2+} release is increased when there is an increase of $[Ca^{2+}]$ in the myoplasm. The increase of $[Ca^{2+}]$ does not increase Ca^{2+} flux.



* Color maps are on different scales.

Conclusion

Our simulation results reveal that:

- 1) The probability that a spontaneous RyR2 opening will trigger a spark is largely dependent on the $[Ca^{2+}]$ in the SR, the probability increased as $[Ca^{2+}]$ increased. The $[Ca^{2+}]$ in the resting myoplasm made insignificant differences.
- 2) The $[Ca^{2+}]$ in the myoplasm have a greater effect on the rate of RyR2 opening than the SR $[Ca^{2+}]$. Increase in myoplasmic $[Ca^{2+}]$ increased the opening rate.
- 3) Leak depends on the $[Ca^{2+}]$ in both the NSR and the myoplasm, but more on the myo plasm.
- 4) The leak was also dependent on the subspace volume. Increasing the volume leads to a greater invisible leak.

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