# Lecture 13
# Systems Biology

Saleet Jafri

## What is Systems Biology?

In traditions science a *reductionist approach* is typically used with an individual system or subsystem is dissected and studied in detail

*Systems biology* integrates information from different sources to understand how larger more complex systems work.

## Systems Biology and Integration

- Molecule (Gene and Protein)
- Organelle (cellular subsystem)
- Cell
- Organ
- Organism
- Environment

## History of System Biology

- The Human Genome Project and modern biotechnology have created the ability to gather large amount of information about an organism.
- Due to the inherent complexity of biological systems, computational methods and models must be used to understand and integrate the data.

## Systems Biology Methods

- There are many methods used in systems biology and each has its strengths and weaknesses
- Much of what is called systems biology relates to modeling genetic networks and biochemical reaction networks, however they are not the only methods.

## Systems Biology Methods

I will present an example from my own research that integrates biochemical, biophysical, and microstructural information to explain the basic mechanisms that initiate contraction in the heart.

# Modeling the Mechanism of Calcium Sparks in the Heart

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# Co-workers

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**Jader Cruz**

## Research Goals

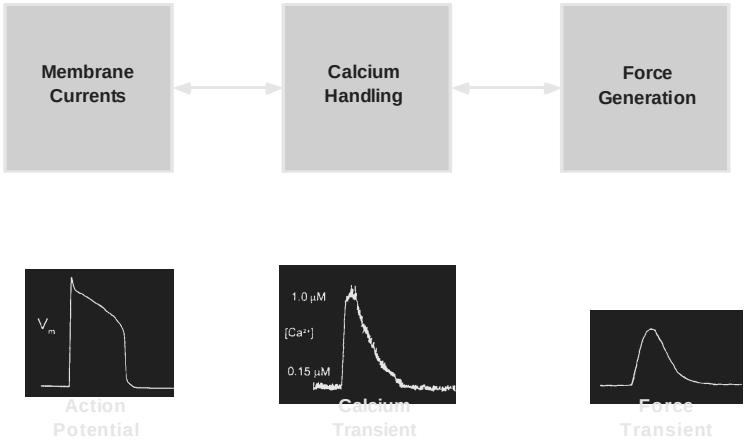
What factors influence spark dynamics?

- What is the mechanism of spark termination?
- How can we account for the spatial spread of the spark?

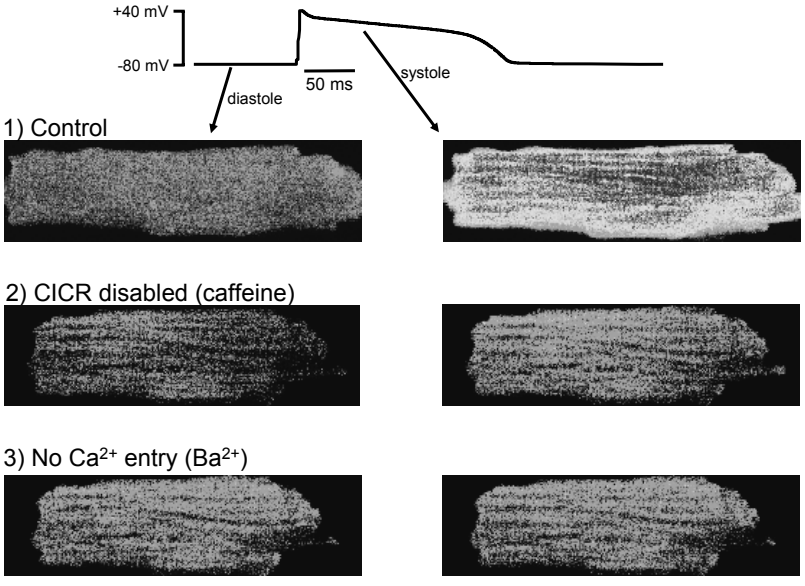
## Presentation Outline

1. Introduction
2. Spark Termination
3. Spatial Spread of Sparks
4. Conclusions

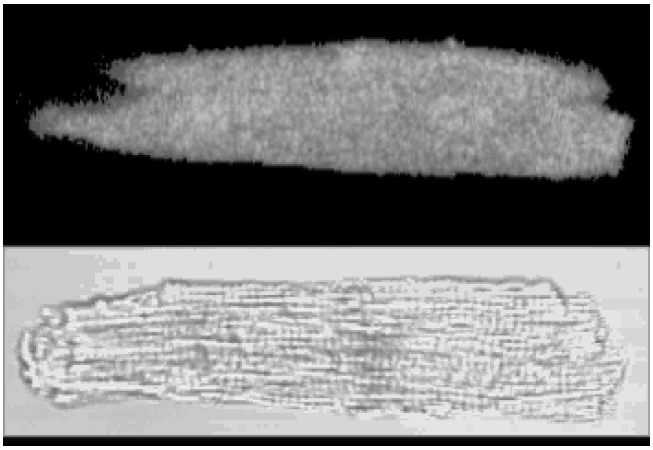
## Basic Components of Cardiac E-C Coupling




## Cardiac $Ca^{2+}$-induced $Ca^{2+}$ release

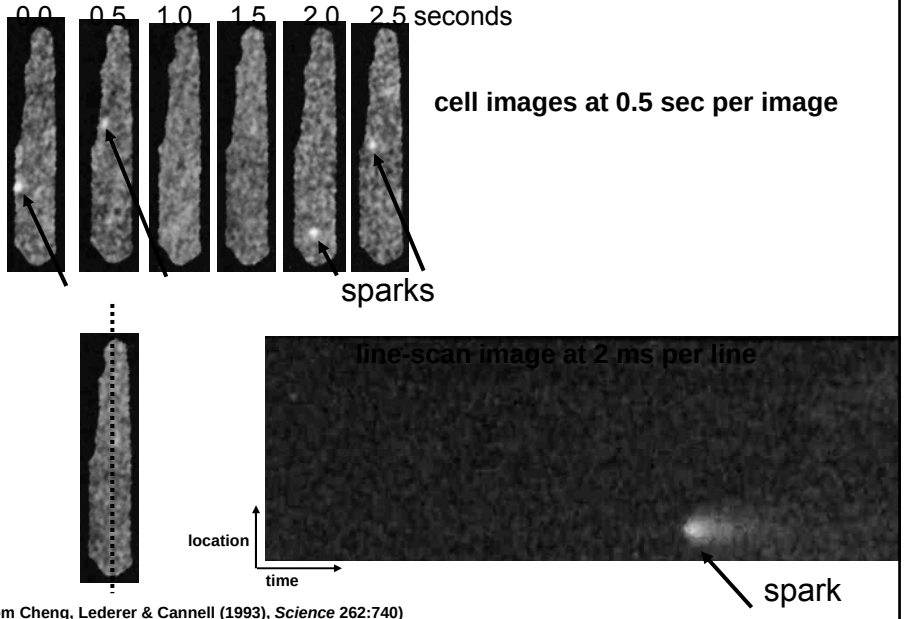


## What is a $Ca^{2+}$ spark?



thanks to Andy Ziman for assistance

## What is a $Ca^{2+}$ spark?




(from Cheng, Lederer & Cannell (1993), *Science* 262:740)

## Why Study Calcium Sparks?

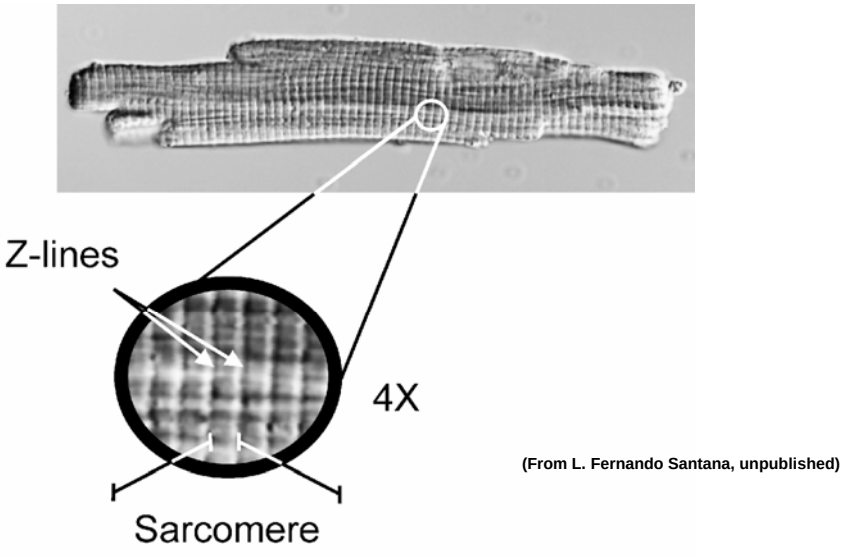
•Calcium sparks are the most elementary observed events in excitation contraction coupling

•Calcium sparks are thought to regulate vascular tone in vascular smooth muscle

•Calcium sparks provide a good example where cellular structure and the detailed biophysics of cellular components combine to observed experimental behavior

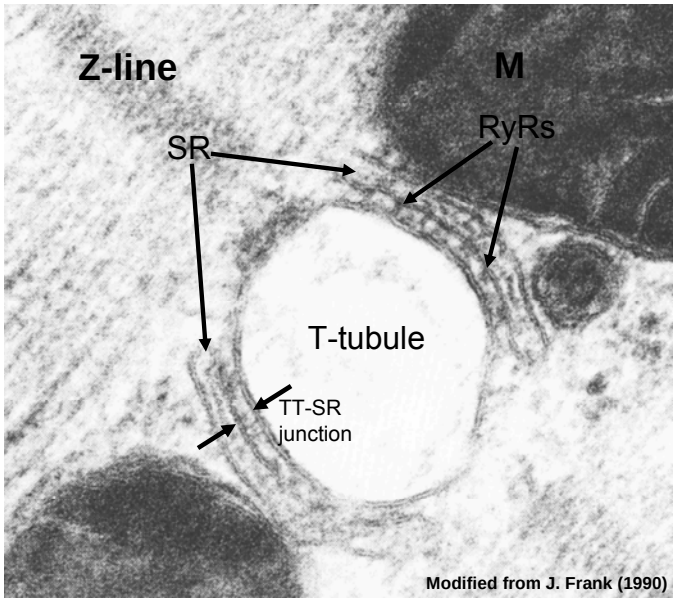
•Excitation contraction coupling is defective in certain diseases such as heart failure.

## Heart Cell

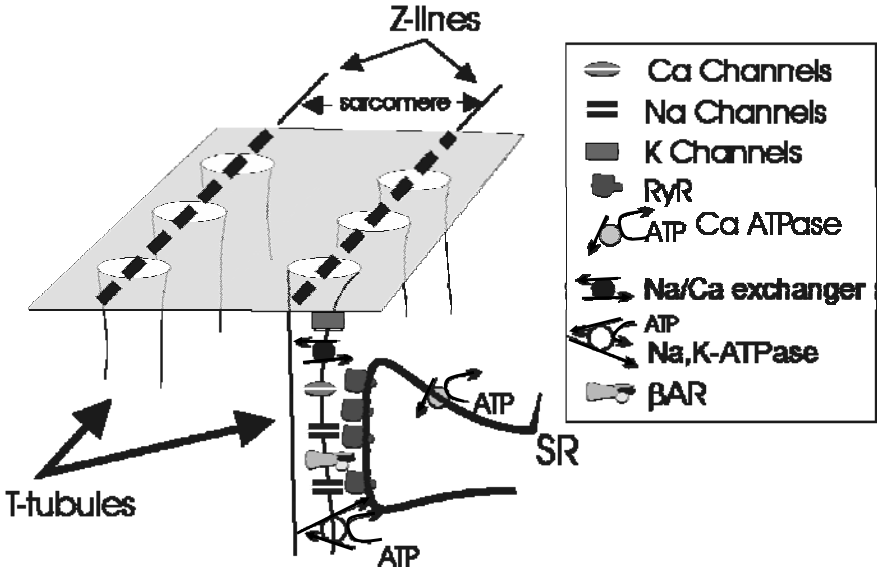



(From L. Fernando Santana, unpublished)

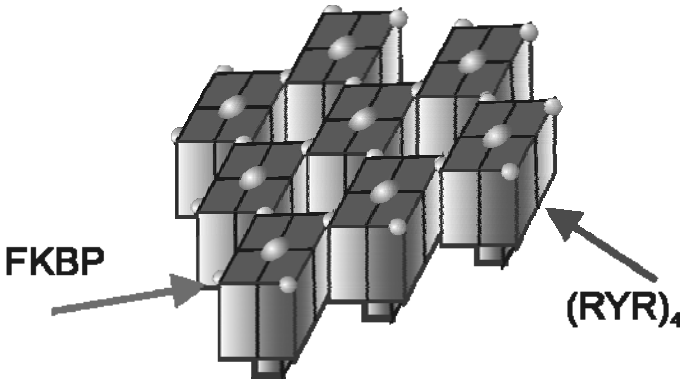
## T-tubules and SR apposition



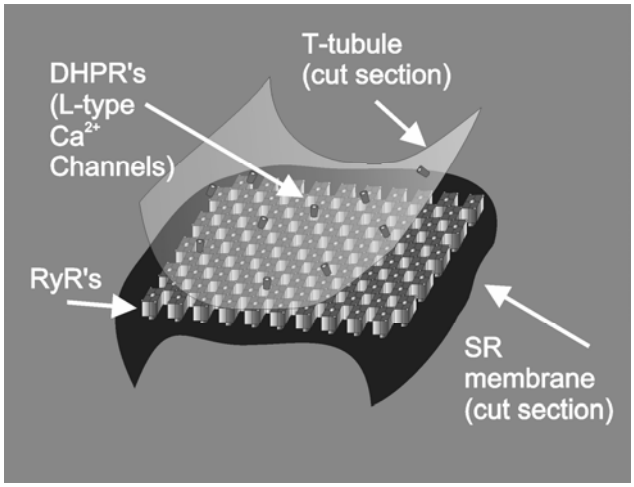

## Elements of $Ca^{2+}$ Spark Generation



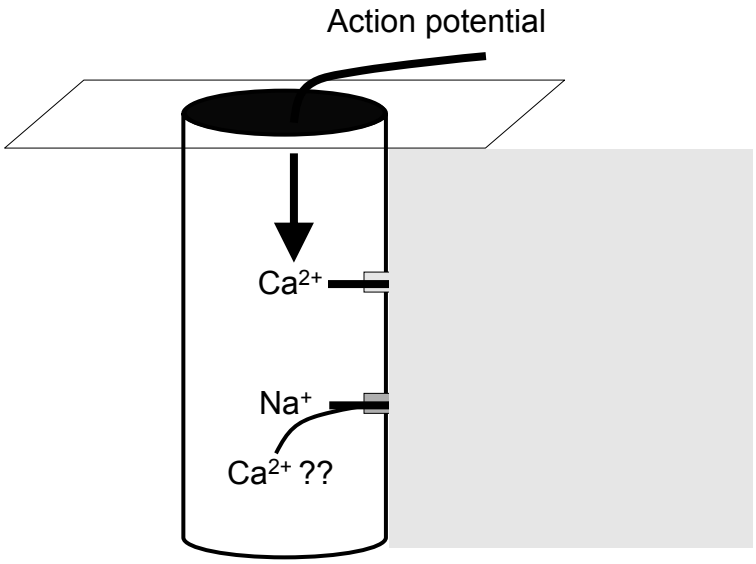
## An array of RyRs




## Junction between T-tubule and the sarcoplasmic reticulum



## Sequence of EC coupling




## Presentation Outline

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4. Conclusions

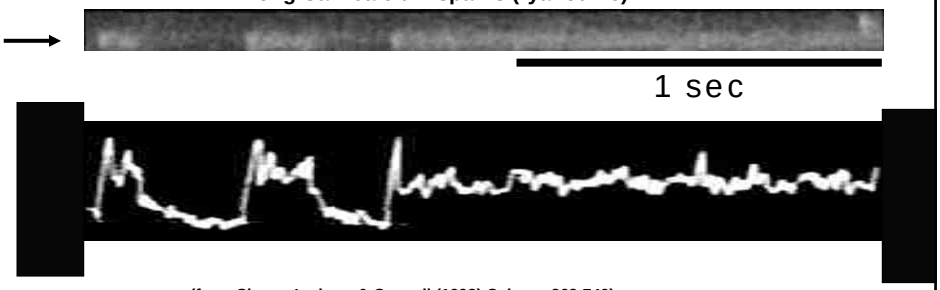
## How do $Ca^{2+}$ sparks terminate?

**Three hypotheses have been proposed to explain the mechanism of $Ca^{2+}$ spark termination:**

1) Depletion of SR $Ca^{2+}$-- do $Ca^{2+}$ sparks terminate because the SR runs out of $Ca^{2+}$?

This is ruled out because – a) There is still Ca available for release after a $Ca^{2+}$ transient (Bassani et al., 1995; Trafford et al., 1997) and b) $Ca^{2+}$ sparks can last a long time – up to seconds.

**long $Ca^{2+}$ calcium sparks (ryanodine)**




**(from Cheng, Lederer & Cannell (1993) *Science* 262:740)**

2) Stochastic attrition?

If $Ca^{2+}$ sparks terminate by "stochastic attrition", it is meant that termination happens when <u>all</u> of the RyR's just happen to close at the same time.

This can occur if there is one or a just a few RyR's, but it is unlikely when the number of RyR's in a cluster is large (e.g. 6 or more) (see analysis by Stern and others, starting with Stern, 1992). In adult heart cells the clusters of RyR's contain 30 or more.

3) Could $Ca^{2+}$ sparks terminate because the RyRs "inactivate"? If not, could "adaptation" do the job?

There are two problems:

- First, "simple inactivation" of RyRs has NOT been observed in planar lipid bilayer experiments.
- Second, adaptation ("complicated inactivation") of RyRs is too slow (100's of ms to seconds). (Gyorke and Fill, 1993; Valdivia et al., 1995)

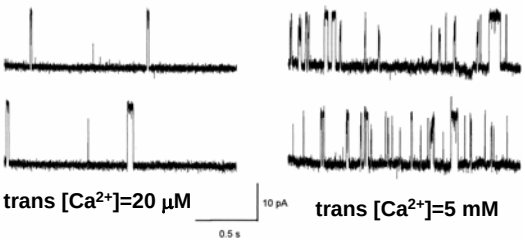
Recent experimental results suggested another hypothesis to us.....

# Hypothesis

$Ca^{2+}$ sparks terminate because of the influence of three factors on RyR gating

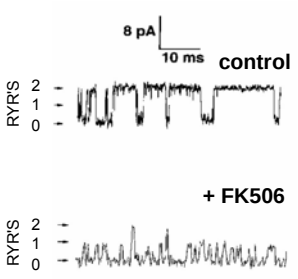
1. Large number of RyRs **(Franzini-Armstrong et al., 1998)**
2. SR lumenal $[Ca^{2+}]$
3. Coupled gating of RyRs

$[Ca^{2+}]_{lumen}$ and RyR gating



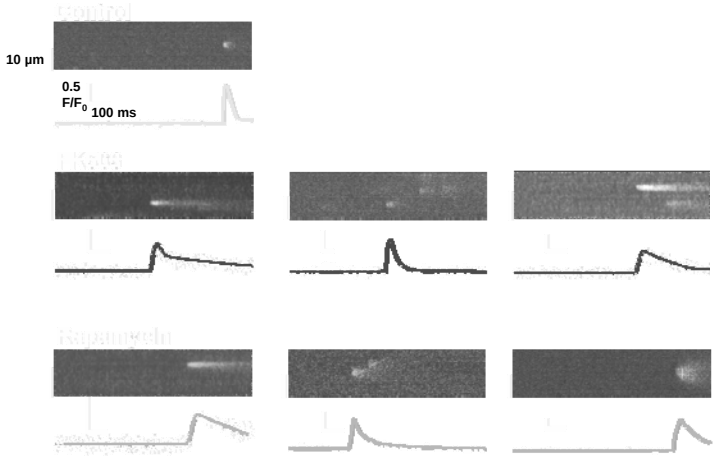

from Gyorke & Gyorke (1998) *Biophys J.* 75:280

Coupled gating of RyRs

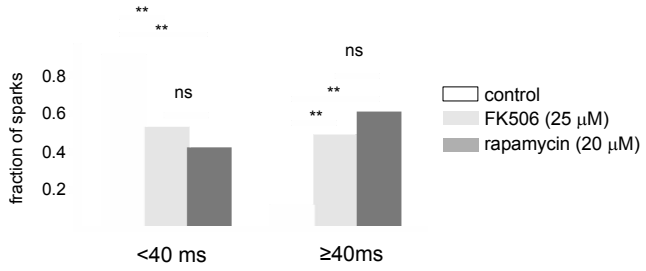



Skeletal Muscle RyRs: Marx et al., (1998) *Science* 281:818.
Heart RyRs: Gaburjakova et al. (2001) *Biophys. J.* 80:380A.

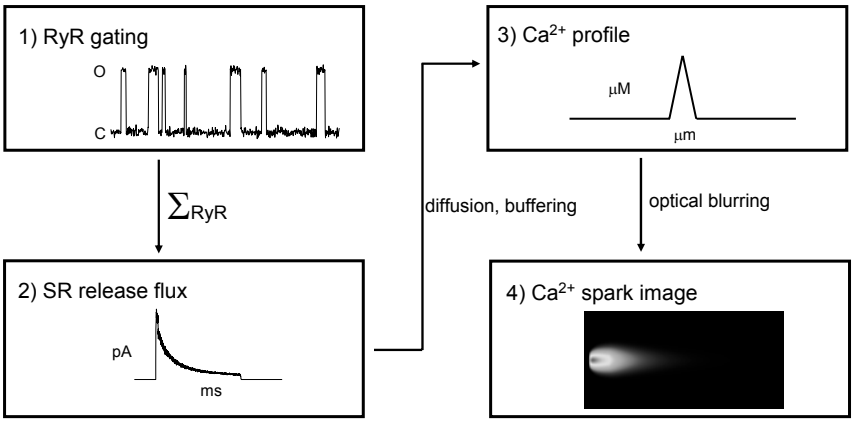
# Experimental Results



# Pooled Experimental Results

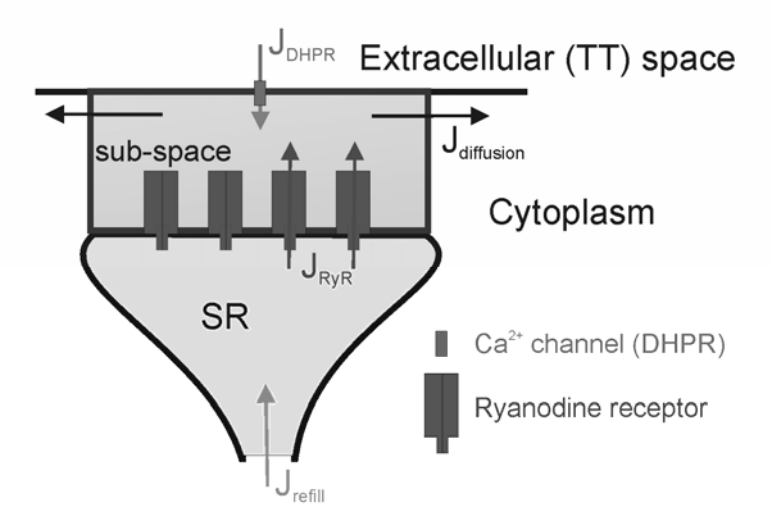



# Model: Conceptual Outline



# Model: "Sticky Cluster"

Spatial organization



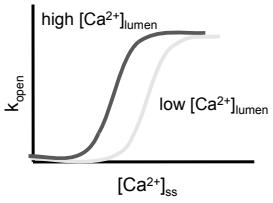

# Model: "Sticky Cluster"

RyR Gating

$$C \underset{k_{close}}{\overset{k_{open}}{\rightleftharpoons}} O$$

$$k_{close} = Const.*CF_{close}$$

$$k_{open} = Const.*CF_{open} \frac{([Ca]_{ss})^4}{K_m{}^4 + ([Ca]_{ss})^4}$$




$$K_m = f([Ca]_{lumen})$$

$$CF_{close} = k_{coop}*g(N_{closed}, N_{open})$$

$$CF_{open} = h(N_{closed}, N_{open})$$

# Model Equations

$$J_{xfer} = \frac{1}{\tau_{xfer}}([Ca^{2+}]_{SS} - [Ca^{2+}]_{Myo})$$

$$J_{tr} = \frac{1}{\tau_{tr}}([Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR})$$

$$J_{RyR} = \sum_{i=1}^{8} J_{RyR} RyR_{open}^{i}([Ca]_{JSR} - [Ca]_{SS})$$

$$J_{DHPR} = \frac{\bar{I}_{DHPR} DHPR_{open}}{2FV_{SS}}$$

where

$$\bar{I}_{DHPR} = \bar{P}_{Ca} 4 \frac{VF^2}{RT} \frac{0.001 e^{2VF/RT} - 0.341[Ca_o]}{e^{2VF/RT} - 1}$$

$$\frac{d[Ca^{2+}]_{SS}}{dt} = J_{DHPR} + J_{RyR} + J_{xfer} + J_{buffer}$$

$$\frac{d[Ca^{2+}]_{JSR}}{dt} = \beta_{JSR}(J_{tr} - \frac{V_{myo}}{V_{SS}} J_{RyR})$$

where

$$J_{buffer} = -k_{on}[Ca^{2+}][B] + k_{off}[CaB]$$

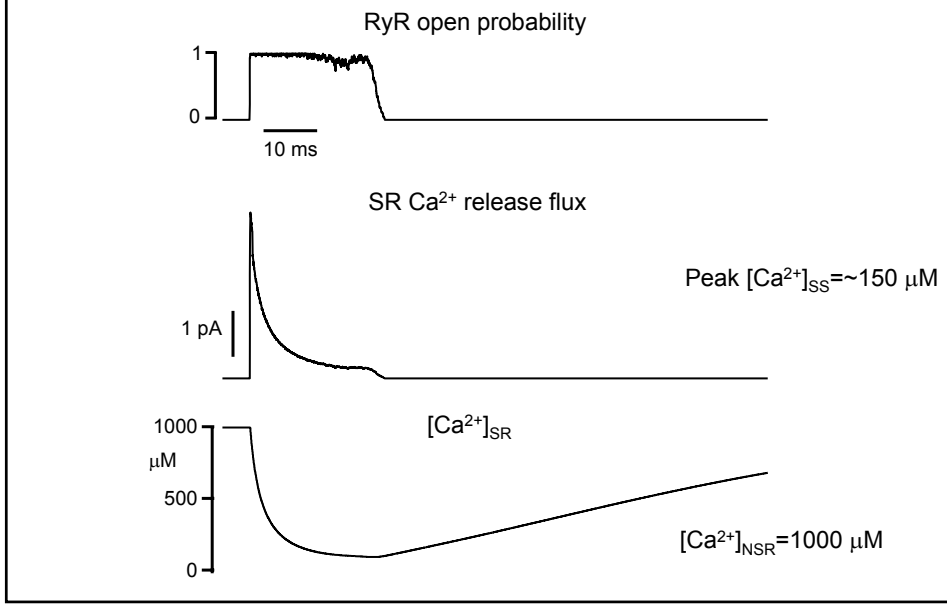
and

$$\beta_{JSR} = \left(1 + \frac{[CSQN]_{total} / K_{CSQN}}{(K_{CSQN} + [Ca^{2+}]_{JSR})^2}\right)^{-1}$$

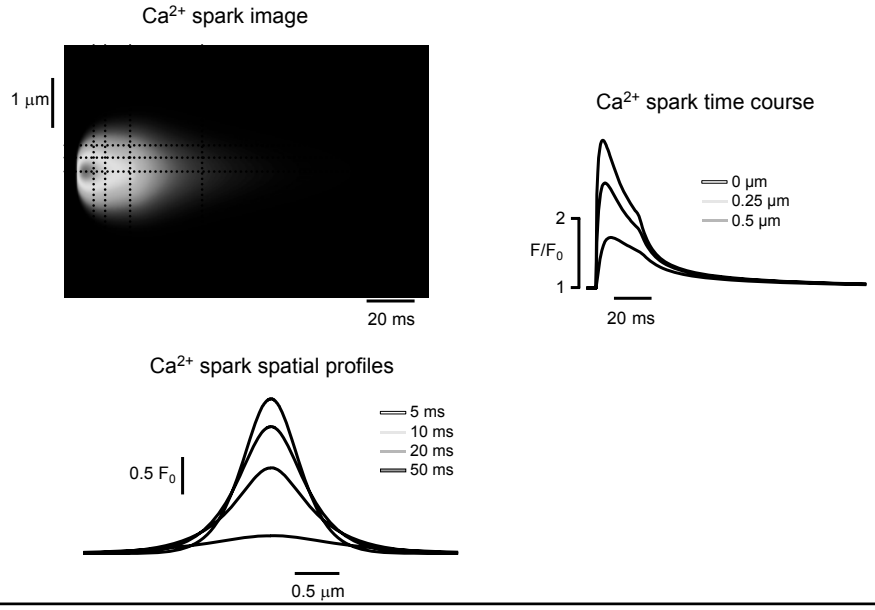
# Model Solution

- RyR open state calculated using a Monte Carlo Method
- Fluxes calculated to determine derivatives
- Differential equations solved using a Euler Method
- Programmed in Fortran 90 on a HP Unix Workstation
- Computation time for control 500 runs in 30 minutes
- Spark visualization determined by solving reaction-diffusion system for buffered diffusion and optical blurring using Matlab on a PC

# Simulated $Ca^{2+}$ release: control conditions

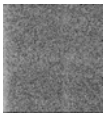



# Simulated $Ca^{2+}$ sparks: control conditions

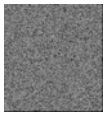


## Simulated $Ca^{2+}$ sparks: effect of noise

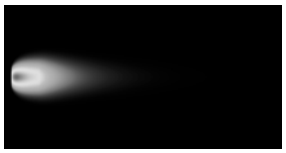
Recorded noise



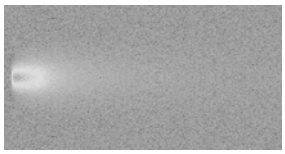
Simulated noise



$Ca^{2+}$ spark image



+ noise =

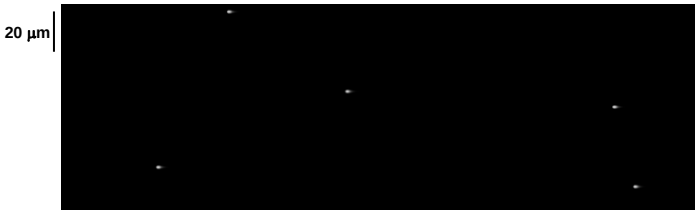


$Ca^{2+}$ spark time course

+ noise =

## Spontaneous simulated $Ca^{2+}$ sparks

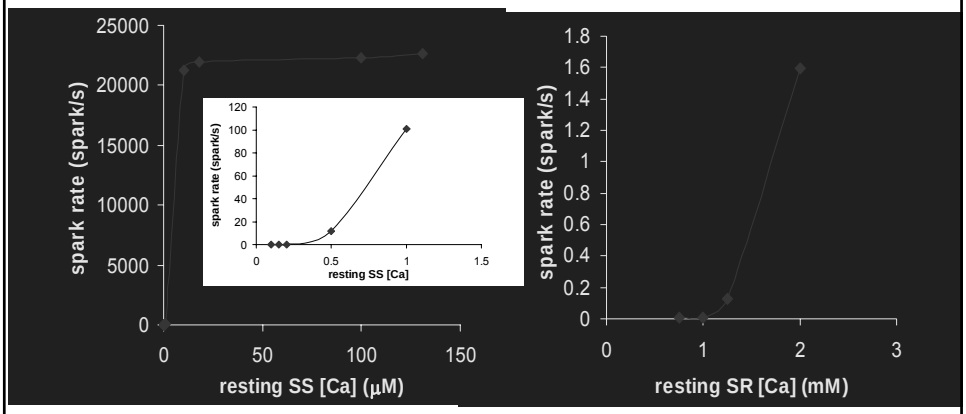
$[Ca^{2+}]_i$ = 100 nM



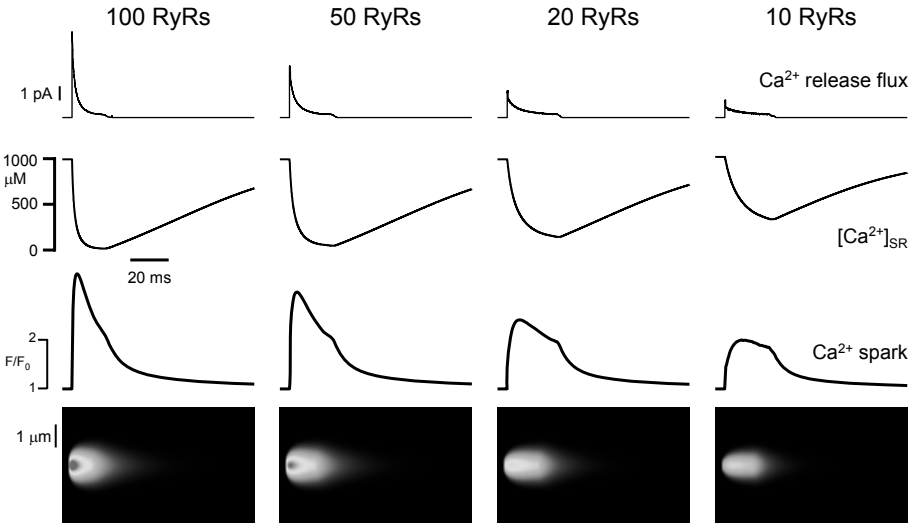

1 second

$[Ca^{2+}]_i$ = 150 nM

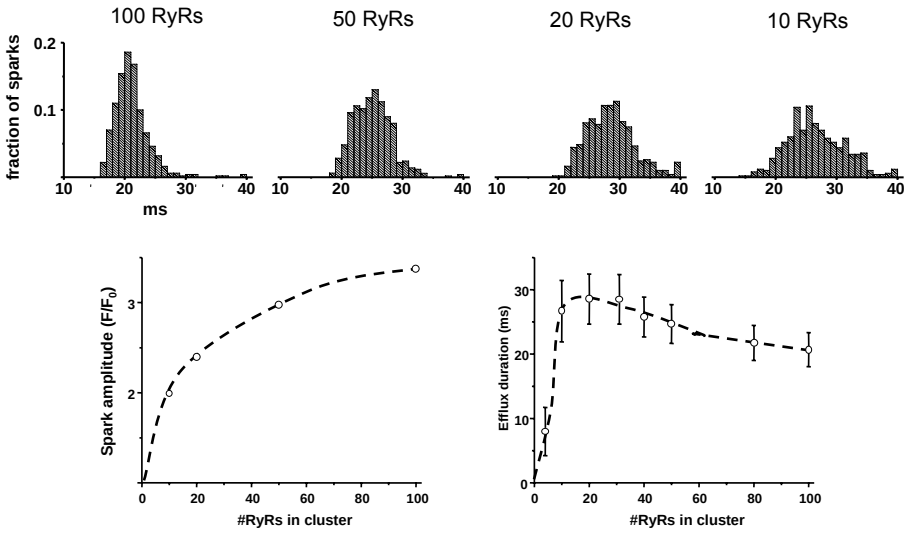
# Spark Rate vs Subspace $Ca^{2+}$ and SR Lumenal $Ca^{2+}$



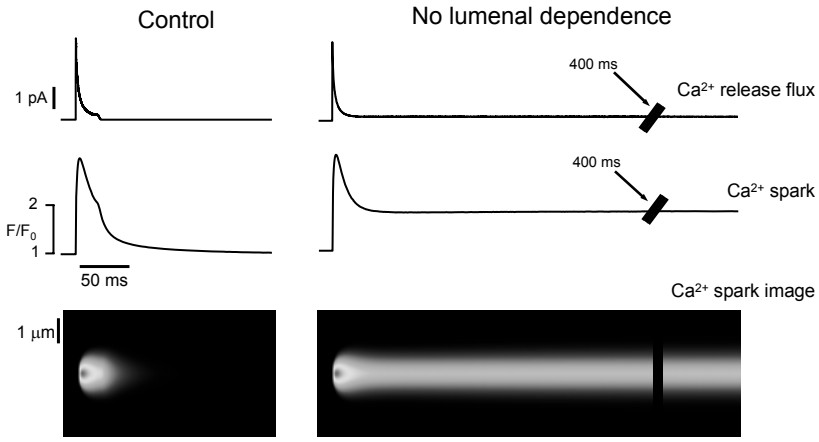

# Simulated $Ca^{2+}$ sparks: cluster size



# Simulated $Ca^{2+}$ sparks: cluster size



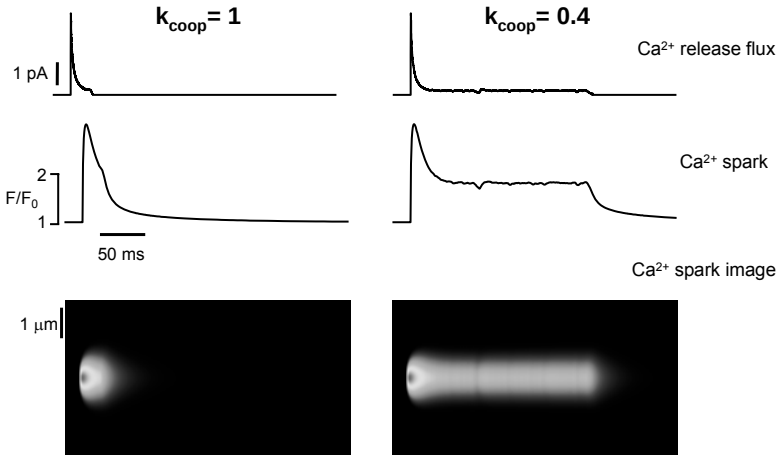

# Simulated $Ca^{2+}$ sparks: no lumenal dependence



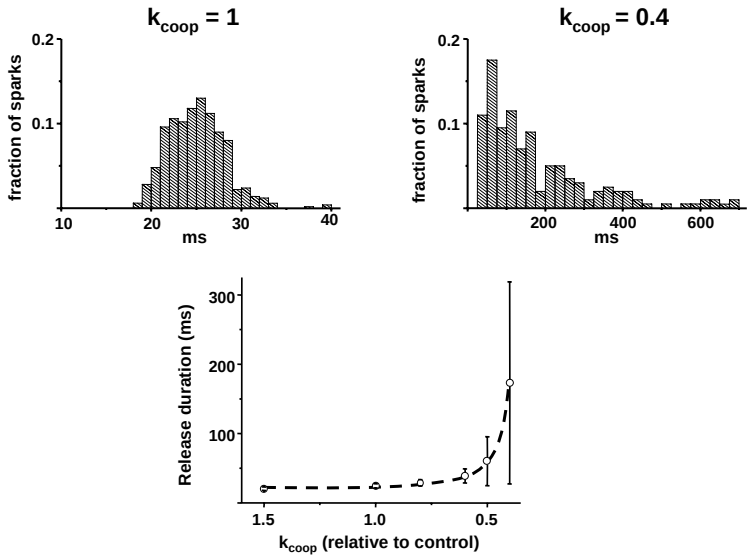
# Simulated $Ca^{2+}$ sparks: SR Load




# Simulated $Ca^{2+}$ sparks: reduced coupling



# Reduced coupling: population data

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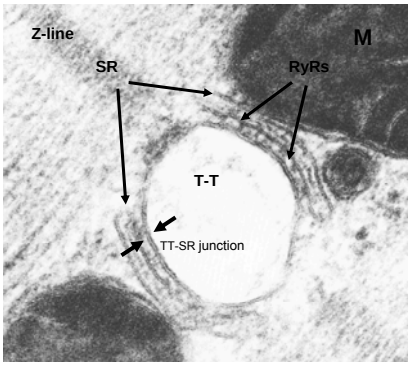
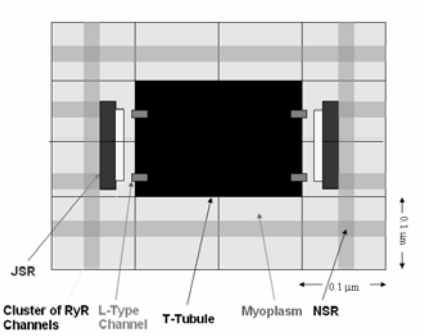
# Involvement of Multiple release sites




Electron micrograph of the t-tubule and SR junction

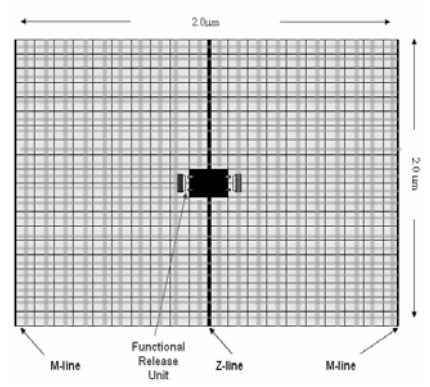
Source: J. Frank 1990




Schematic diagram of the model TT-SR junction

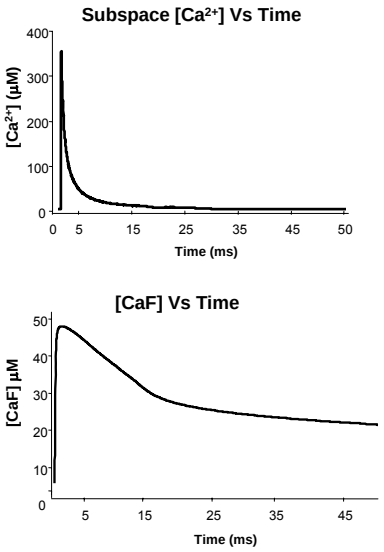
- Conventionally sparks are thought to originate from a signal release site
- Parker et al suggest that sparks can originate from multiple release sites depending on the proximity of the release site

# Model Layout and Method



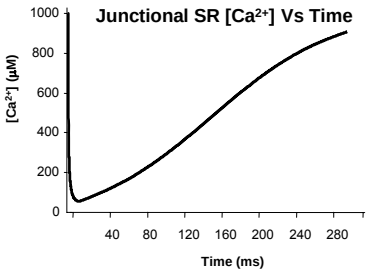

- Model Layout
  - Two Functional Release Units
    - 2 LCC
    - 32 RyR Channel
  - Homogeneous NSR and Myoplasm
- Method
  - Explicit finite difference method (Euler Method)
    - Time step of $10^{-7}$ s
    - Spatial step of 0.1 µm
  - Monte Carlo method to determine RyR channel state
  - No flux boundary conditions

# Calcium Dynamics in Functional Release Unit

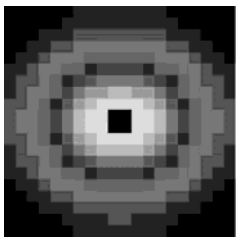




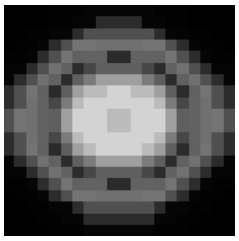




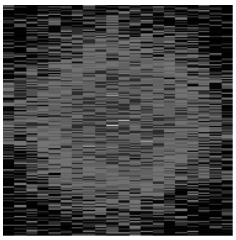

# Undistinguished Calcium Spark Peaks



[CaF]



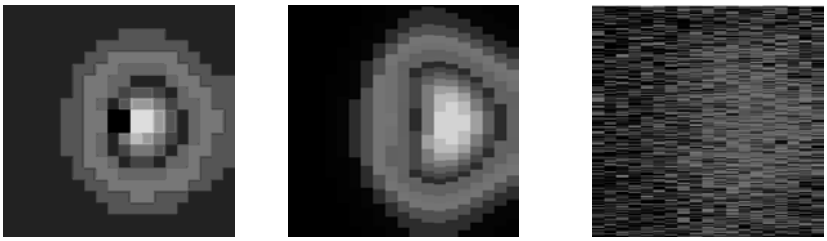
[CaF] with Optical blurring



Simulated Spark

- Release from one site almost always triggers release from the adjacent site on the other side of the T-tubule consistent with the results of Parker et al. 1996.
- FWHM = 2.0 μm

# Spark from One Release Site

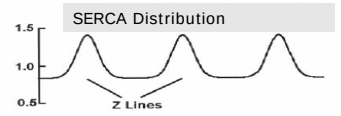


**[CaF]** **[CaF] with Optical blurring** **Simulated Spark**

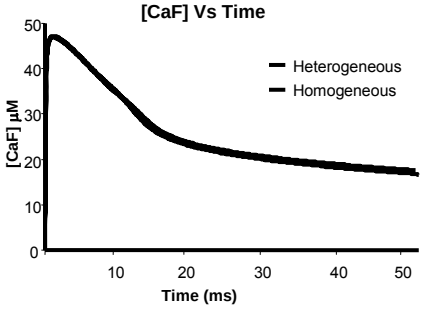
- One site is disables to show release from a single site
- FWHM < 2.0 μm

# SERCA Pump Distribution

Distribution



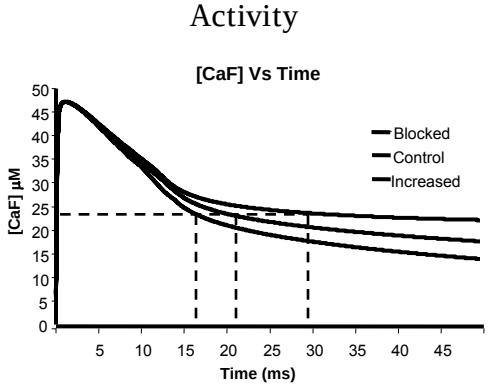

Smith et al. 1998



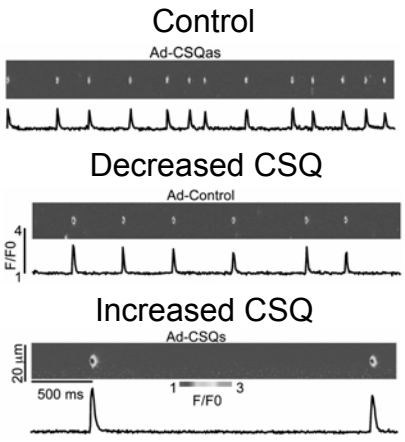

- Periodic distribution predicted by Smith and co-workers
- Homogeneous and non-homogeneous SERCA pump distribution made little difference on spark duration

# SERCA Pump Activity

- Increasing SERCA pump activity leads to decrease spark duration
- Blocking SERCA pump activity leads to an increase in spark duration similar to that observed by Gomez et al 1996.



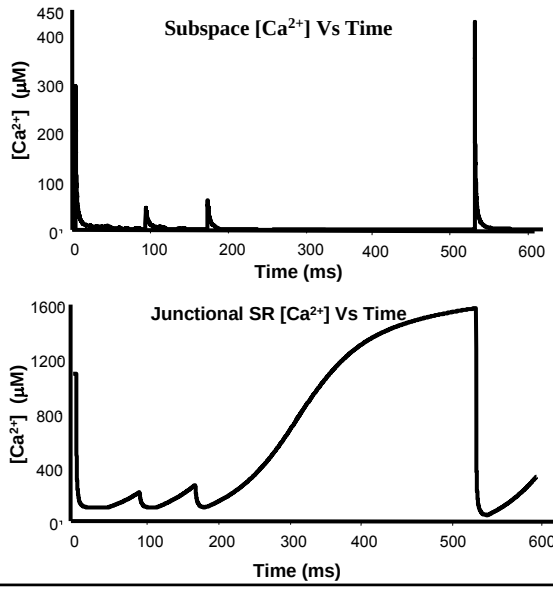

# Calsequestrin and Sparks




Terentyev et al., 2003

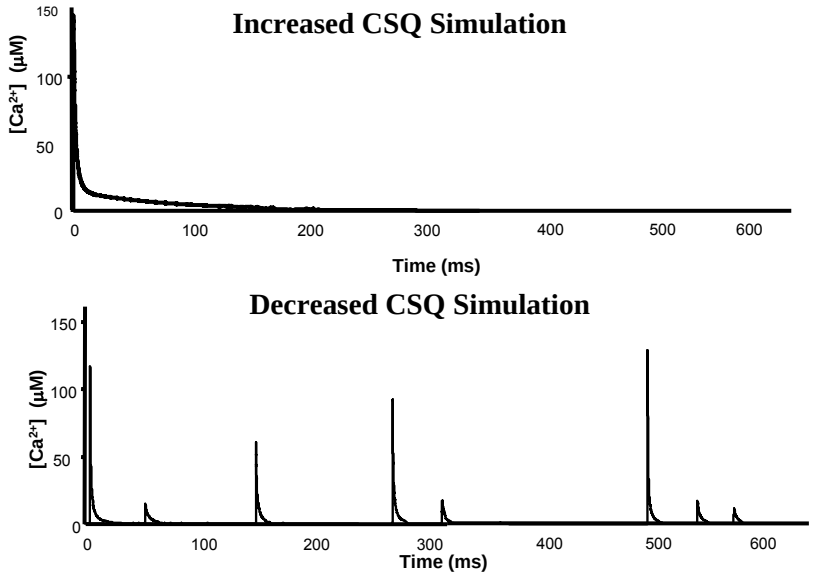
- Iperatoxin was addedto cardiac myocytes to increase spontaneous sparks from the same site.
- Decreased calsequestrin expression increases spark frequency
- Increased calsequestrin expression decreases spark frequency.

# Spark Restitution




- Spark amplitude increases as interspark interval increases
- The lower spark amplitude is a result of the partially filled state of the SR
- Since Popen depends on $[Ca^{2+}]_{SR}$ the iperatoxin results can be explained by a delay in refilling.

# Simulated Effects of Calsequestrin



# SR Buffer Data

| Condition | Peak Amplitude | Peak duration | %Spark rate (Sim.) | %Spark rate (Exp.) |
|---|---|---|---|---|
| Control | 128 μM | 24 ms | 100% | 100% |
| Increased CSQ expression | 147 μM | 150 ms | 28% | 27% |
| Decreased CSQ expression | 120 μM | 18 ms | 190 % | 183% |
| Citrate | 156 μM | 170 ms | 27% | 38% |

Terentyev et al., 2003

# Presentation Outline

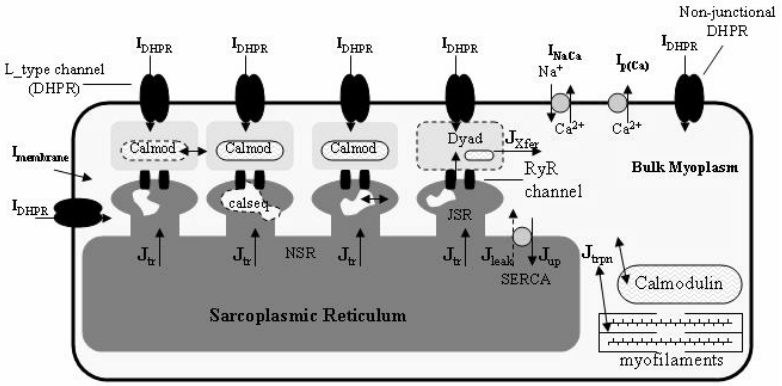
1. Introduction
2. Spark Termination
3. Spatial Spread of Sparks
4. Conclusions

# Summary/Conclusions

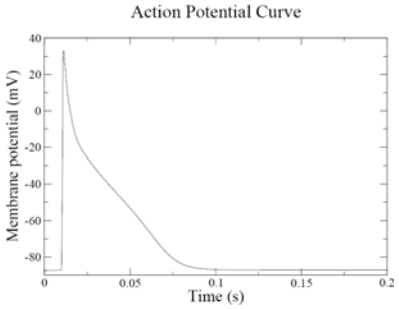
- Our “sticky cluster” model of a $Ca^{2+}$ release unit can simulate $Ca^{2+}$ sparks that terminate reliably. Termination occurs through coupled gating and the influence of lumenal calcium.
- Reducing coupling between RyRs increases $Ca^{2+}$ spark duration, consistent with experimental effects of FK506.
- $Ca^{2+}$ spark magnitude is only mildly sensitive to the number of RyR's in the cluster and the $Ca^{2+}$ spark duration is even less sensitive to this number.
- Release from adjacent sights might combine to give spark widths of 2 μm as observed experimentally.
- The spontaneous spark rate alteration due to SR buffers is likely due to their effect on refilling of the SR.

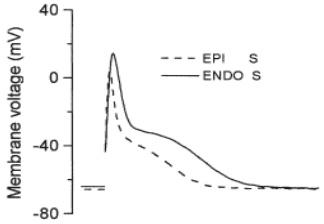
# Current Work

- We have integrated the $Ca^{2+}$ spark model into a model for whole cell $Ca^{2+}$ dynamics of the cardiac myocyte to demonstrate that the summation of many sparks from different release sites give rise to the global $Ca^{2+}$ transient.



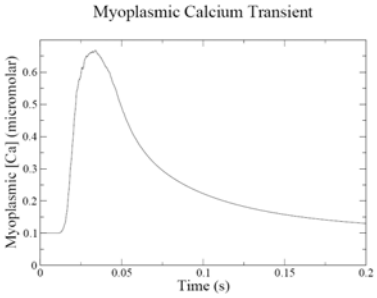
# Cardiac Myocyte Results



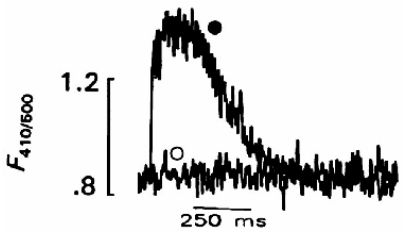




***AP curve Obtained from Natali et al. 2002***

# Cardiac Myocyte Results

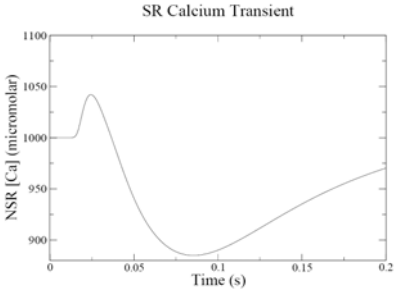


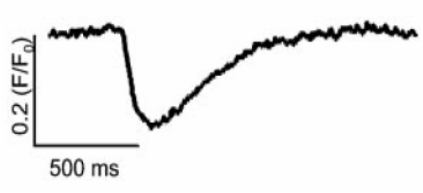

*Simulated patch clamp experiments under normal conditions. 20,000 FRUs simulated.*




***Transient recordings by Bouchard et al. 1995***

# Cardiac Myocyte Results

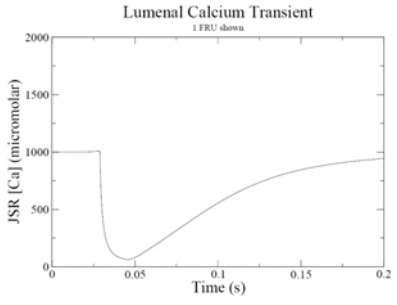


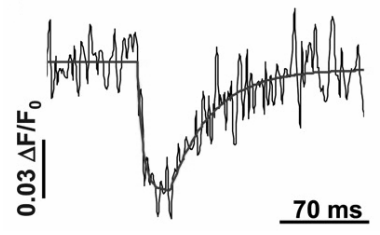




***SR transient recorded by Wang et al. 2004***

*Simulated patch clamp experiments under normal conditions. 20,000 FRUs simulated.*

# Cardiac Myocyte Results

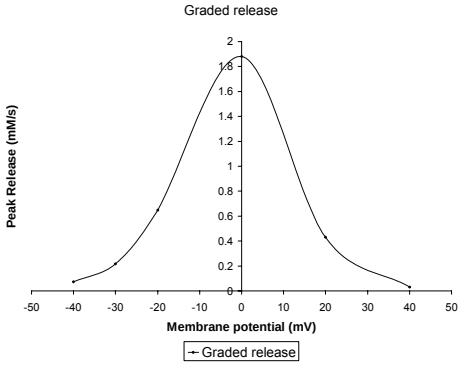


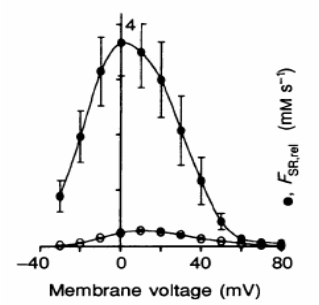

***Lumenal transient recorded by Brochet et al. 2005***

*Simulated patch clamp experiments under normal conditions. 20,000 FRUs simulated.*

# Cardiac Myocyte Results

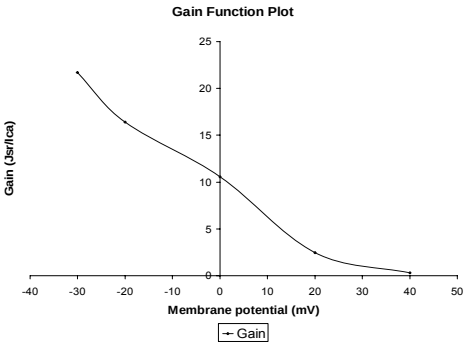



*Simulated patch clamp experiments under normal conditions. 20,000 FRUs simulated.*

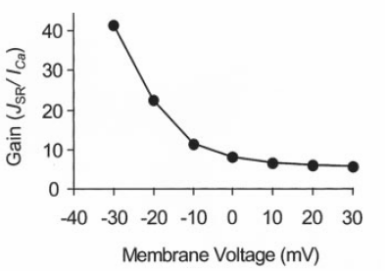



***Release curve recorded by Wier et al. 1994***

# Cardiac Myocyte Results

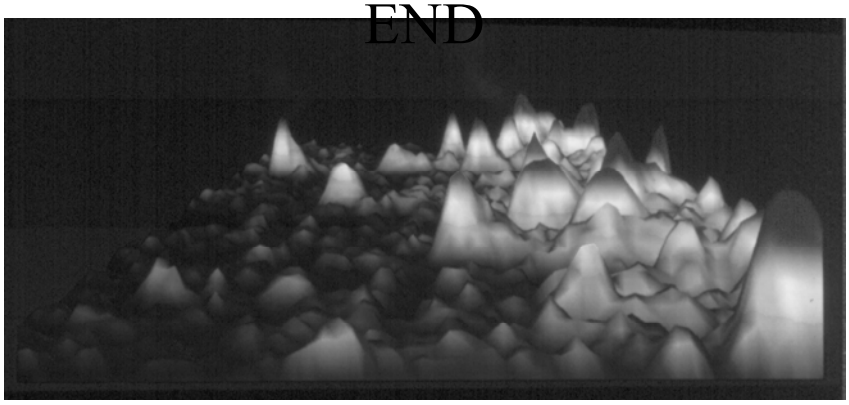



*Simulated patch clamp experiments under normal conditions. 20,000 FRUs simulated.*




***Experimentally generated gain plot from Wang et al. 2004***

END



**$Ca^{2+}$ sparks activated during ramp-depolarization from -60 to -40**

**(from Cannell, Cheng & Lederer (1995), *Science* 268: 1045.)**