Cardiac Excitation-Contraction Coupling

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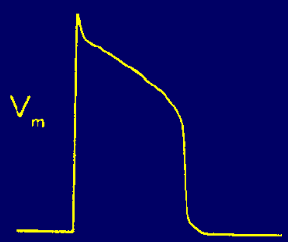
Presentation Overview

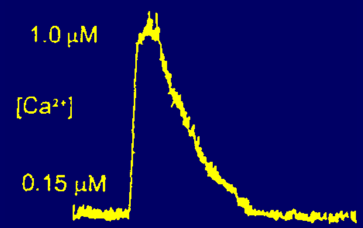
- Introduction
  - Excitation Contraction Coupling
  - Graded Release
  - Morphology and Function
- Common Pool Models
- Calcium Sparks
- Local Control
- Heart Failure

Basic Components of Cardiac E-C Coupling

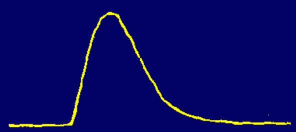
Membrane Currents ⟷ **Calcium Handling** ⟷ **Force Generation**



Action Potential



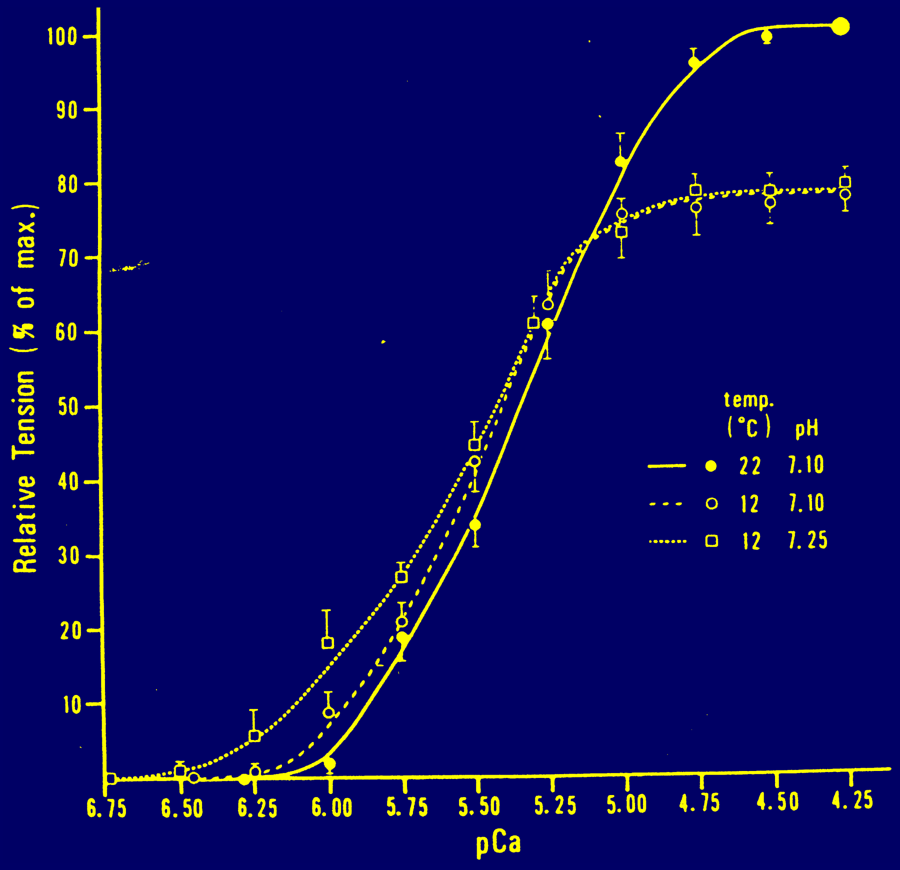
Calcium Transient



Force Transient

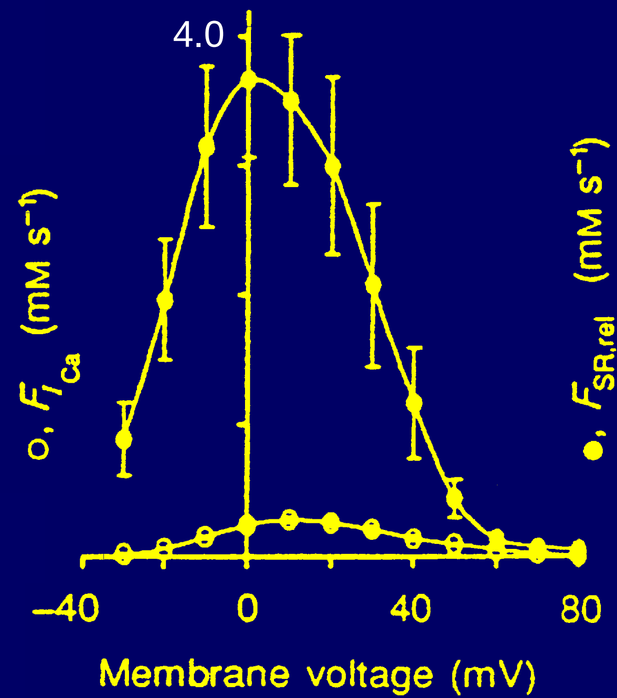
Graded Release in Skinned Cardiac Myocytes

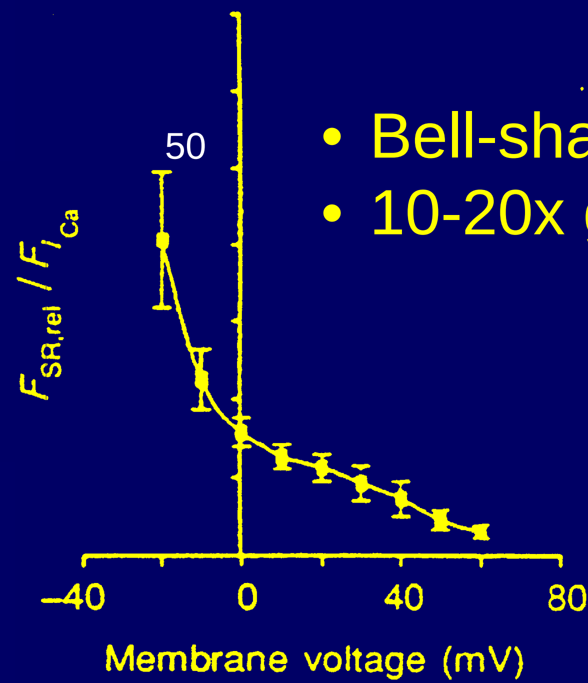
- As $[Ca^{2+}]$ increases, so does relative tension



Reprinted from Fabiato, 1985

Graded Release in Rat Cardiac Myocytes

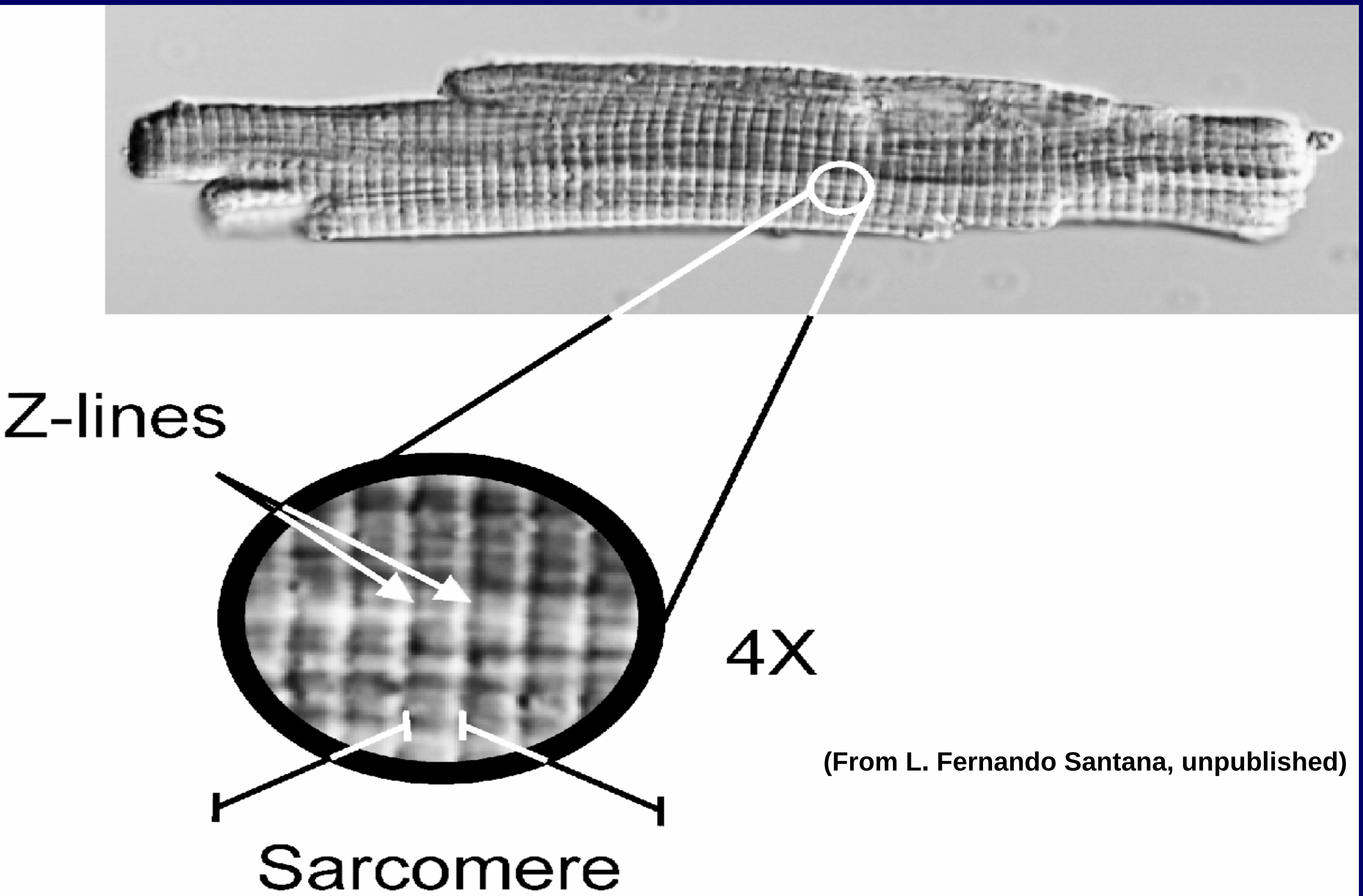




- Bell-shaped curve
- 10-20x gain at 0 mV

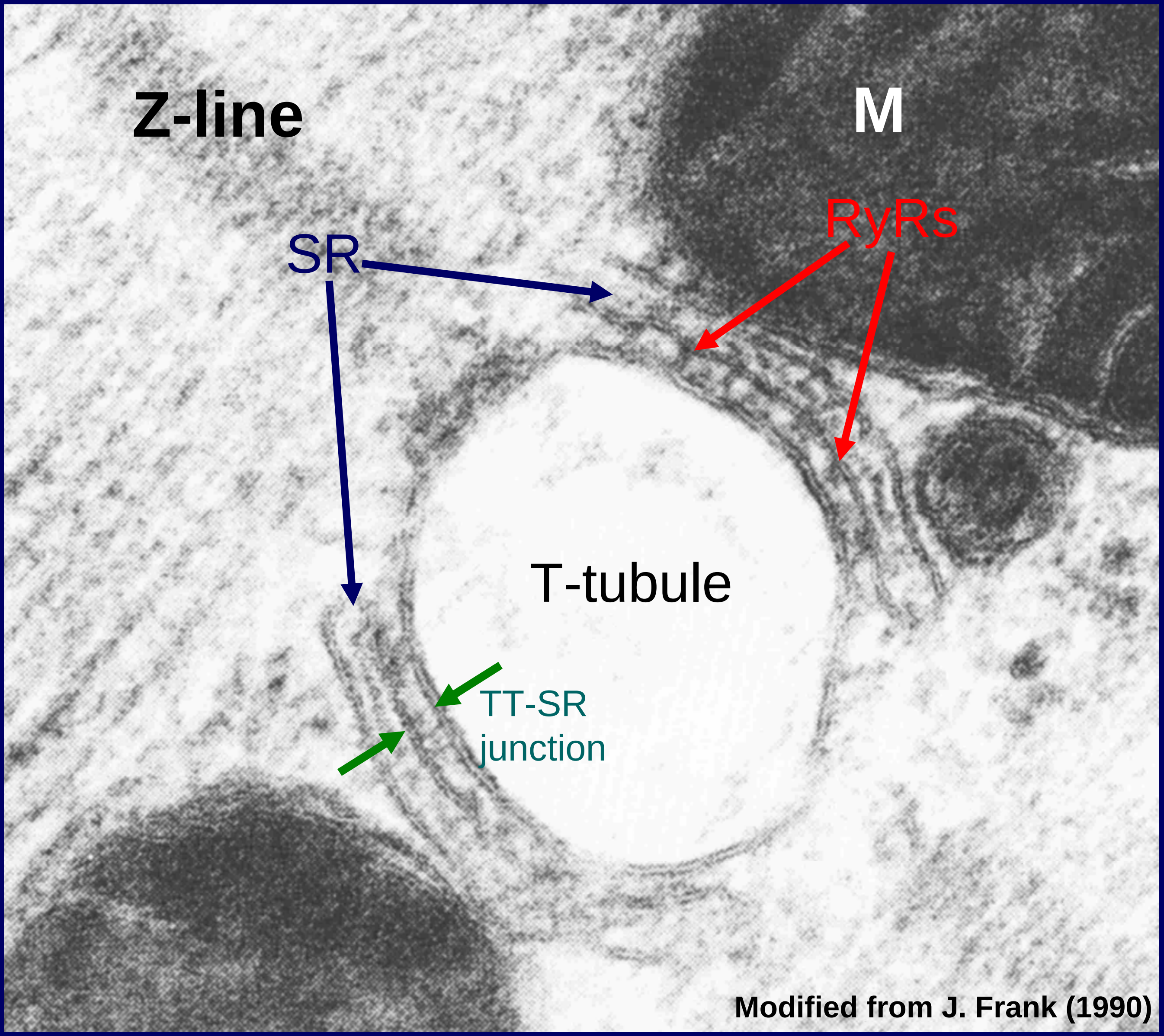
Wier, et al., 1994

Heart Cell

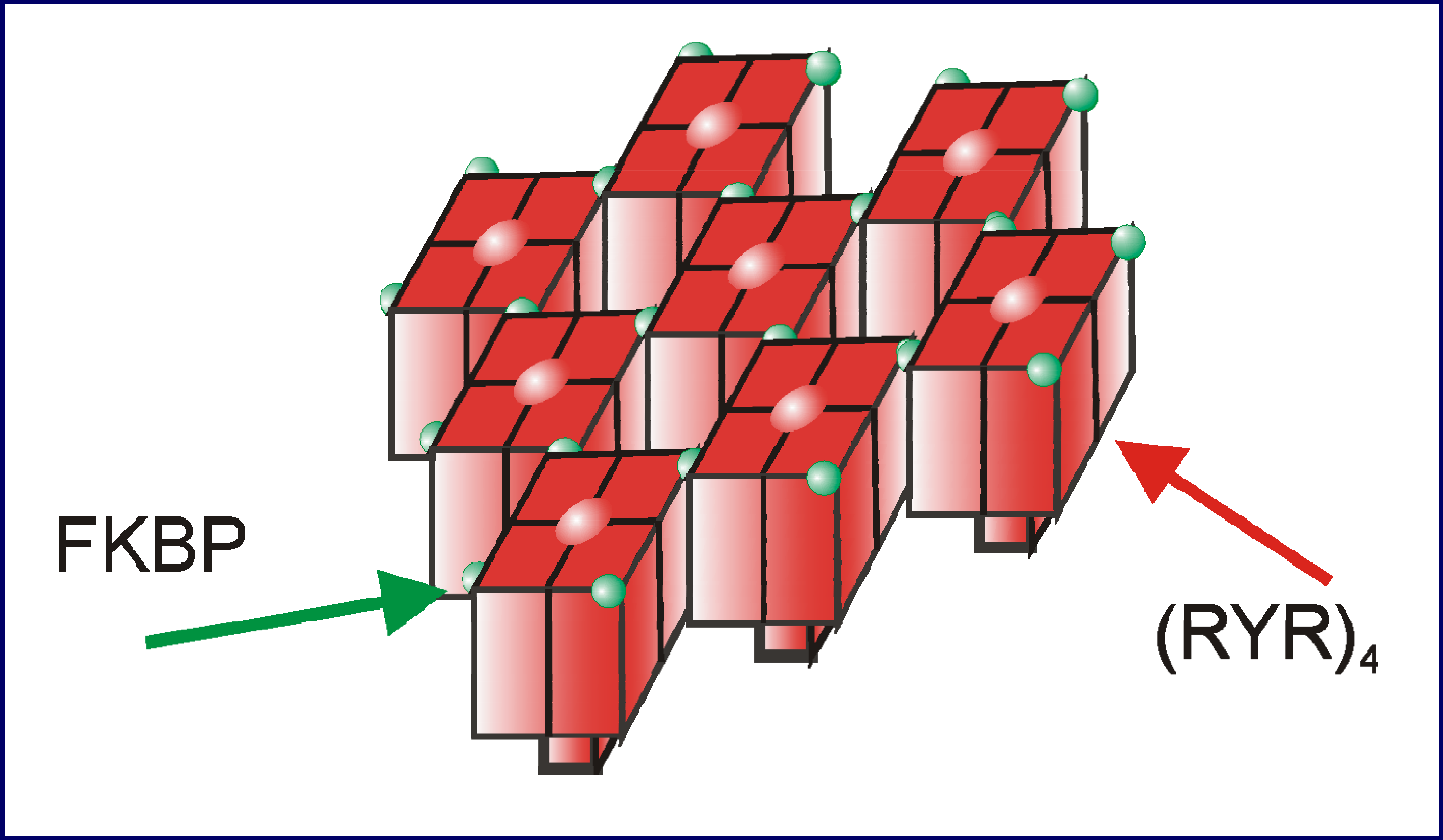


(From L. Fernando Santana, unpublished)

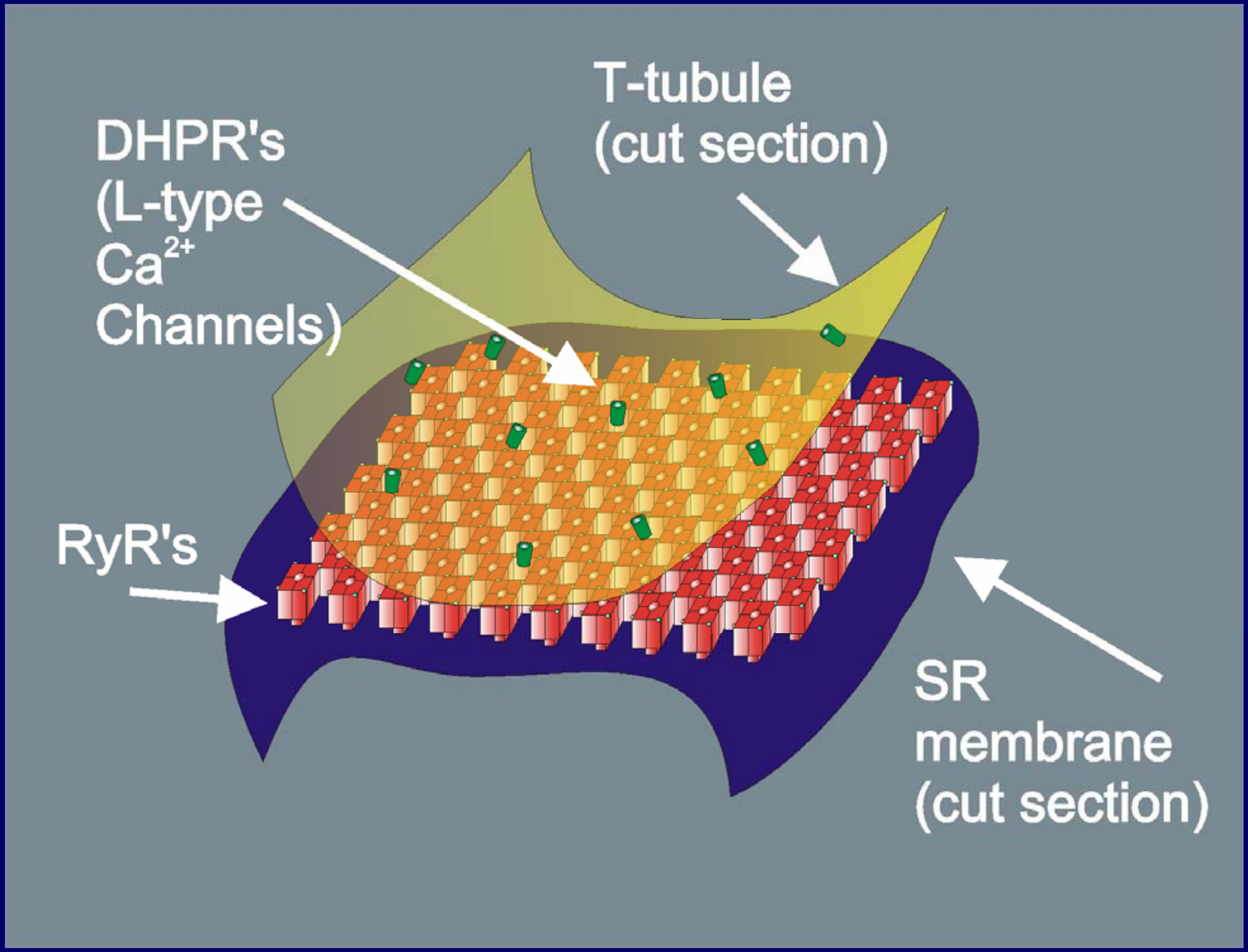
T-tubules and SR apposition



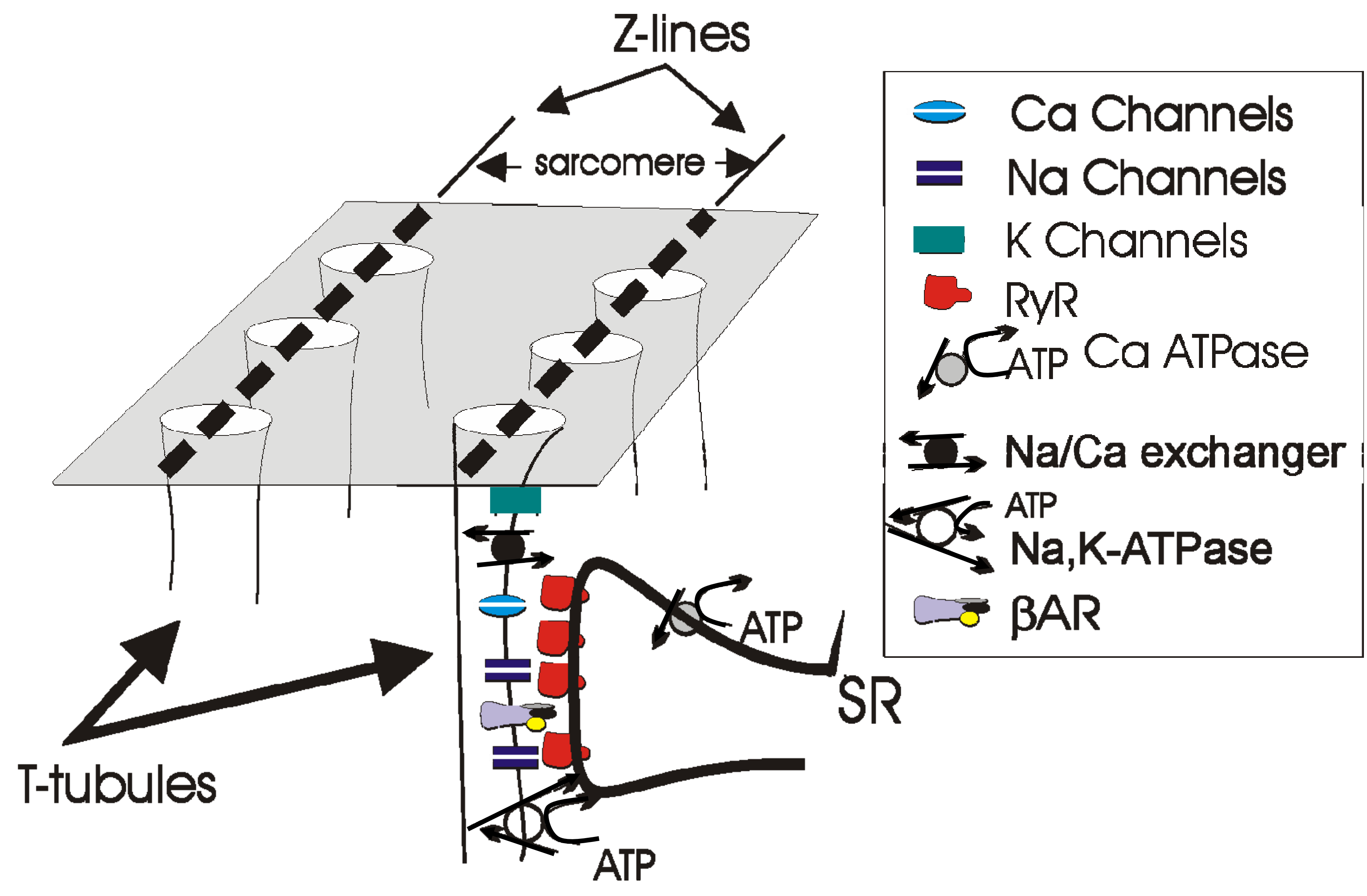
Diadic RyR Arrangement



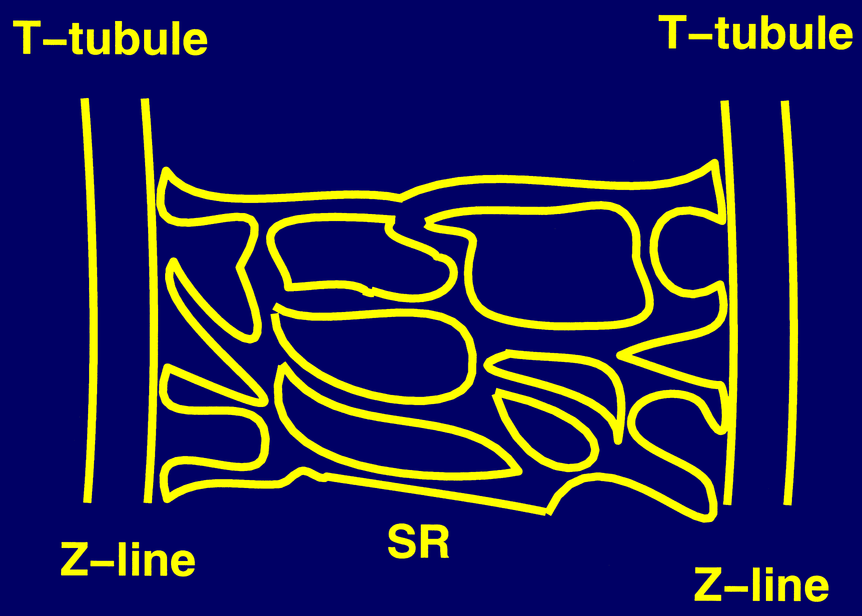
Junction between T-tubule and the sarcoplasmic reticulum



Elements of Ca^{2+} Spark Generation



Sarcomere Geometry

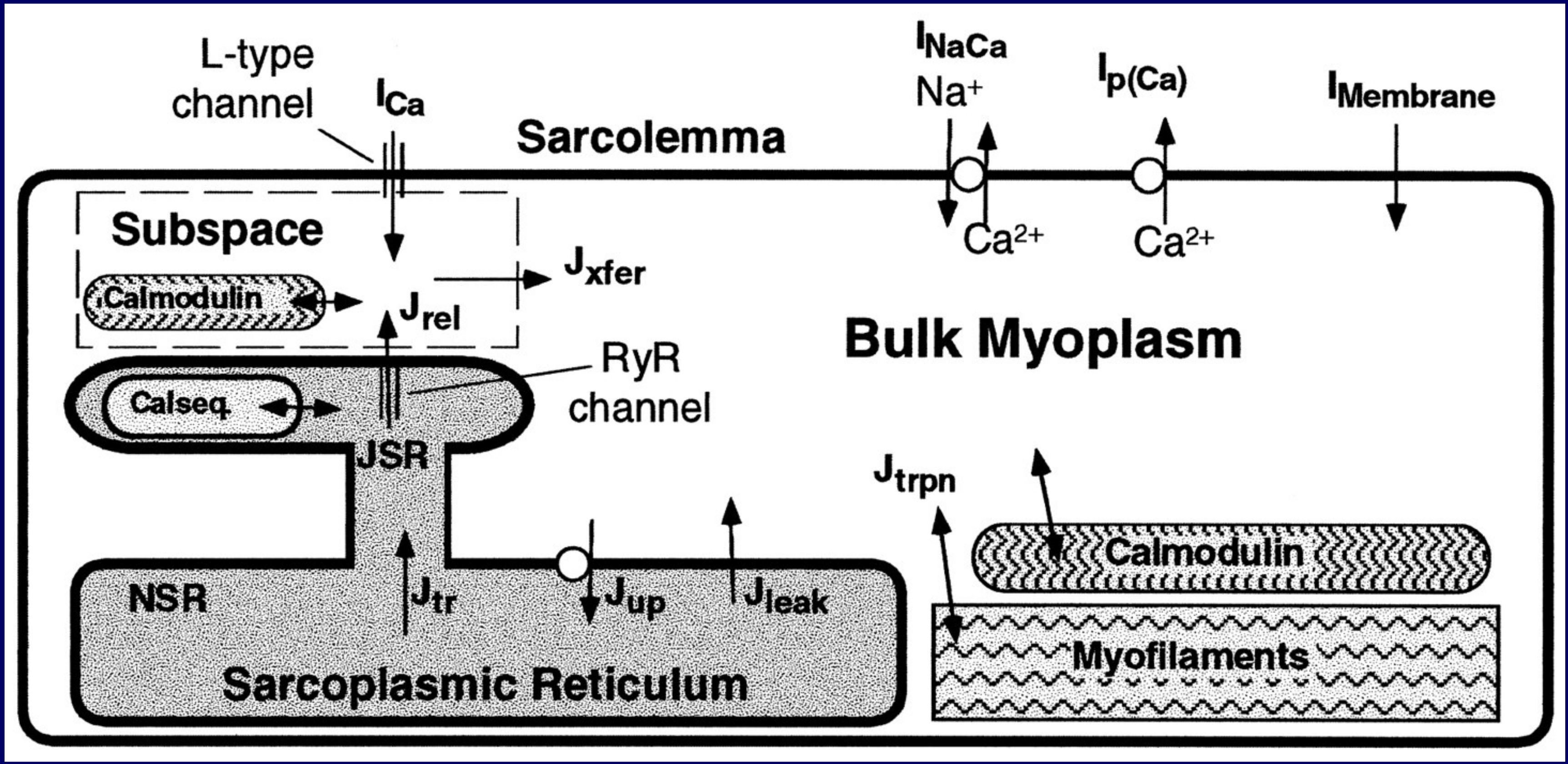


- Many diads
- Diads separated
- NSR connections
- All sarcomeres shorten uniformly

Presentation Overview

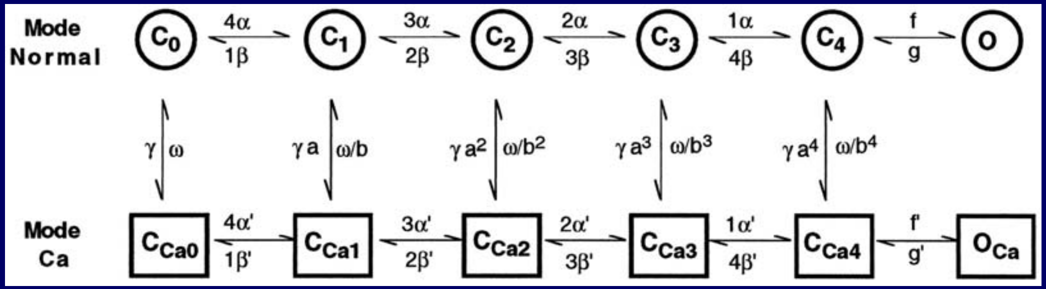
- Introduction
- Common Pool Models
  - How does calcium release terminate?
  - What is the role of adaptation?
- Calcium Sparks
- Local Control
- Heart Failure

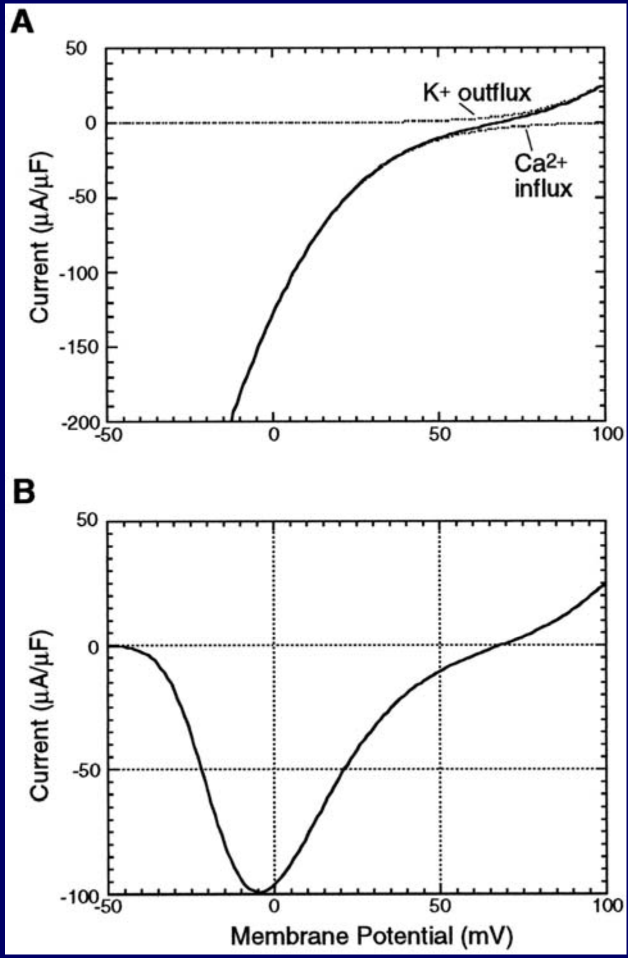
Jafri-Rice-Winslow Model



Jafri, Rice, and Winslow, 1998

L-type Calcium Channel



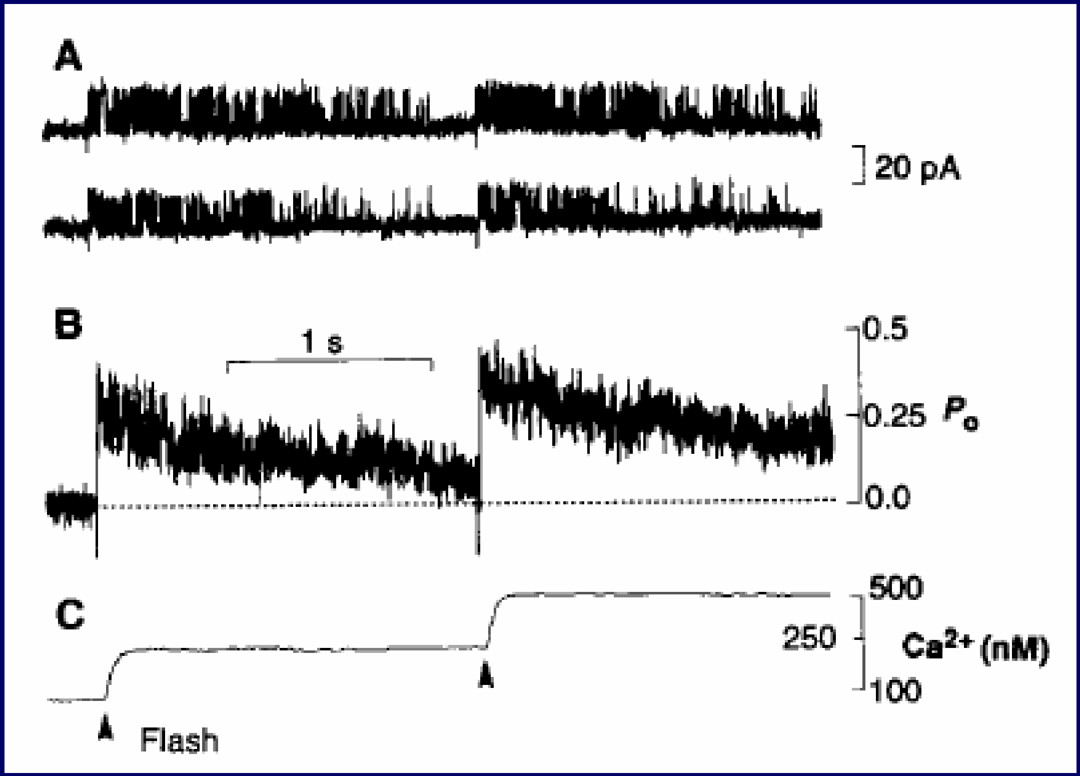


- **Markov state Model**
- **Matches experimental data**

Jafri, Rice, and Winslow, 1998

Adaptation

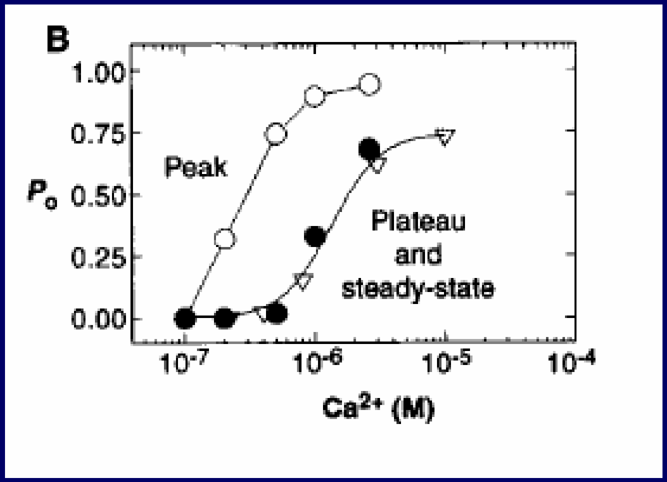
- Flash photolysis of caged Ca^{2+} results in increased open probability that adapts.
- The channels can reopen with another increase in Ca^{2+}.

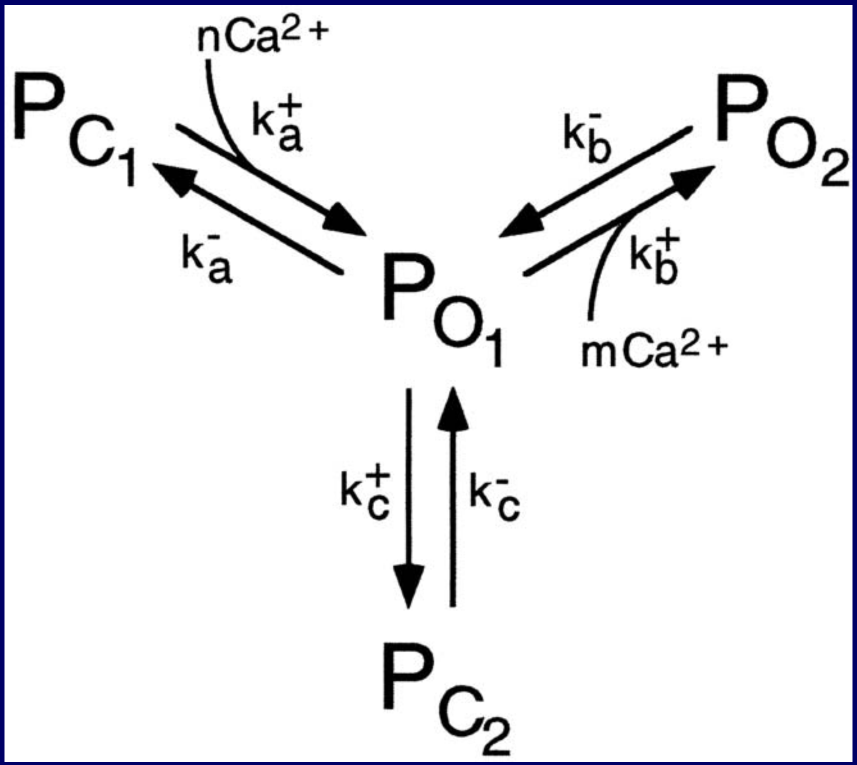


Gyorke and Fill, 1993

Ryanodine Receptor

- Keizer and Levine Model
- Adaptation
- Open and close probabilities and dwell time





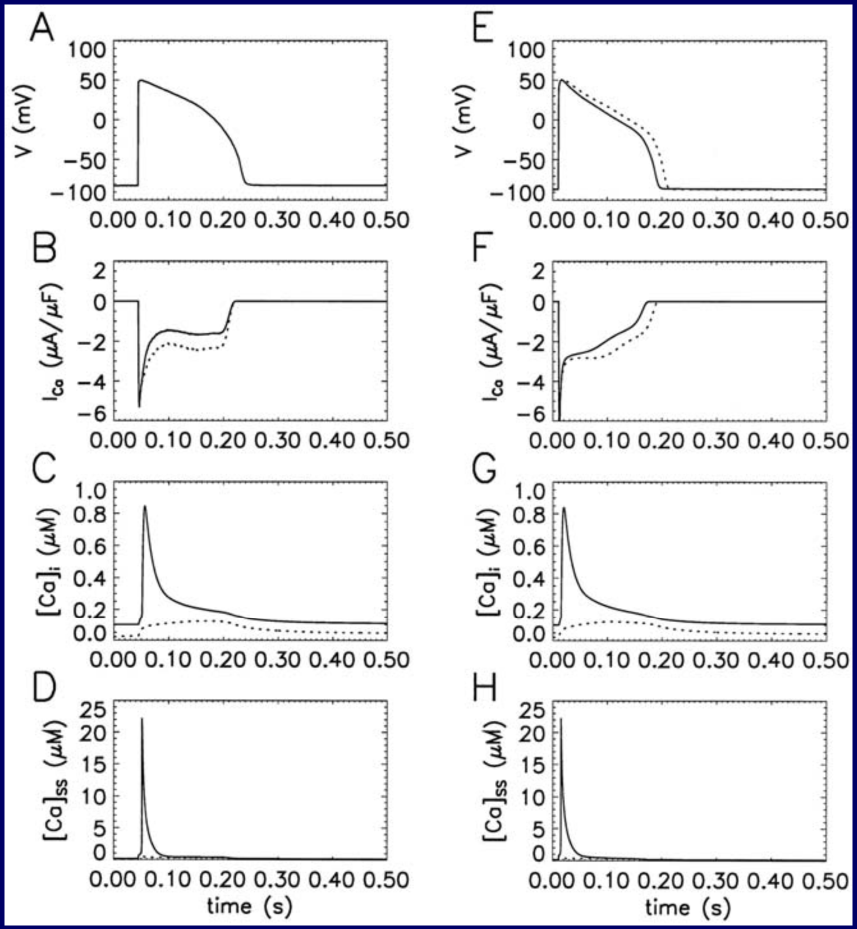
Keizer and Levine, 1996

Gyorke and Fill, 1993

Model Results

A.-D. Action potential clamp (Grantham and Cannell)

E.-H Model Results



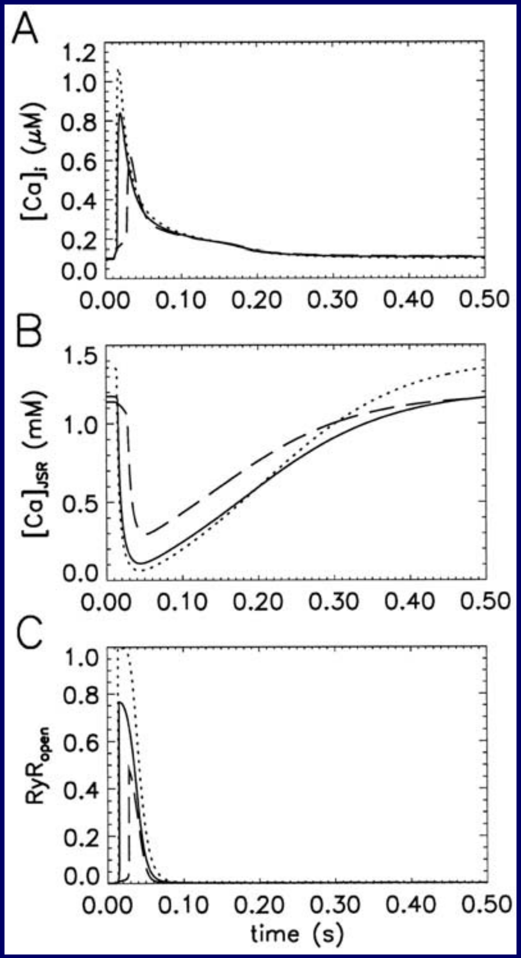
Jafri, Rice, and Winslow, 1998

Adaptation and Termination

Control (solid)

10x increased adaptation rate (dashed)

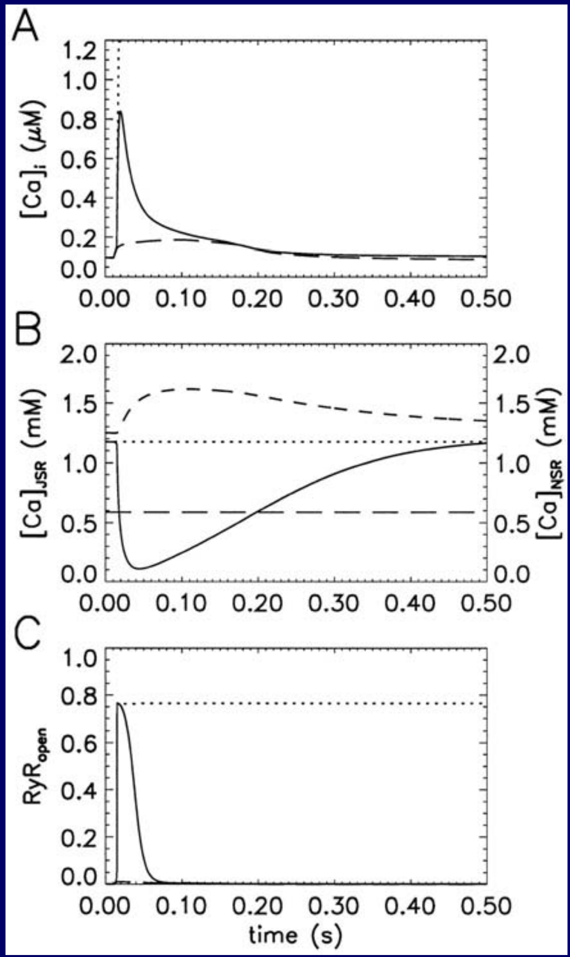
10X decreased adaptation rate (dotted)



Jafri, Rice, and Winslow, 1998

SR Depletion and Termination

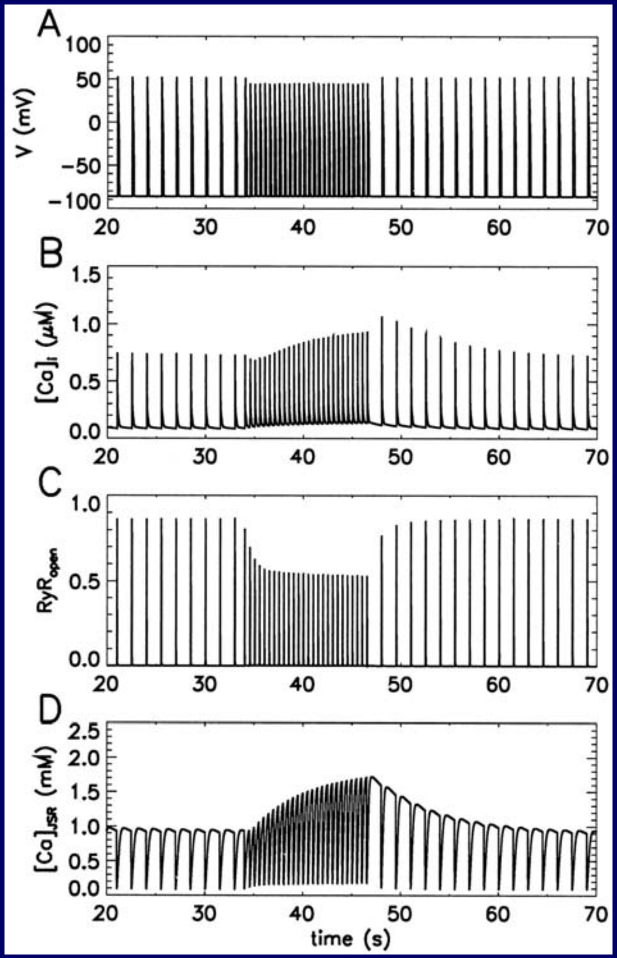
- If SR is held fixed at its full value, release does not terminate (dotted).
- If the SR is held fixed and a depleted value above its minimum little release (dashed)
- There is still available calcium to release, but the flux out of the RyR is not self-sustaining



Jafri, Rice, and Winslow, 1998

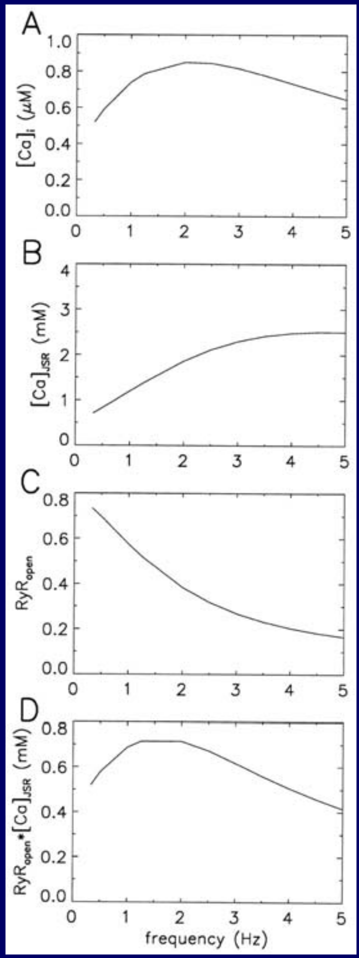
Interval-force relations

- Changing pacing frequency changes force generated as indicated by Ca^{2+} transients.
- RyR peak open probability increases with increasing pacing rate
- SR fills with increased pacing rate
- $J_{rel}=D_{RyR}P_{open}([Ca^{2+}]_{SR} - [Ca^{2+}]_{SS})$



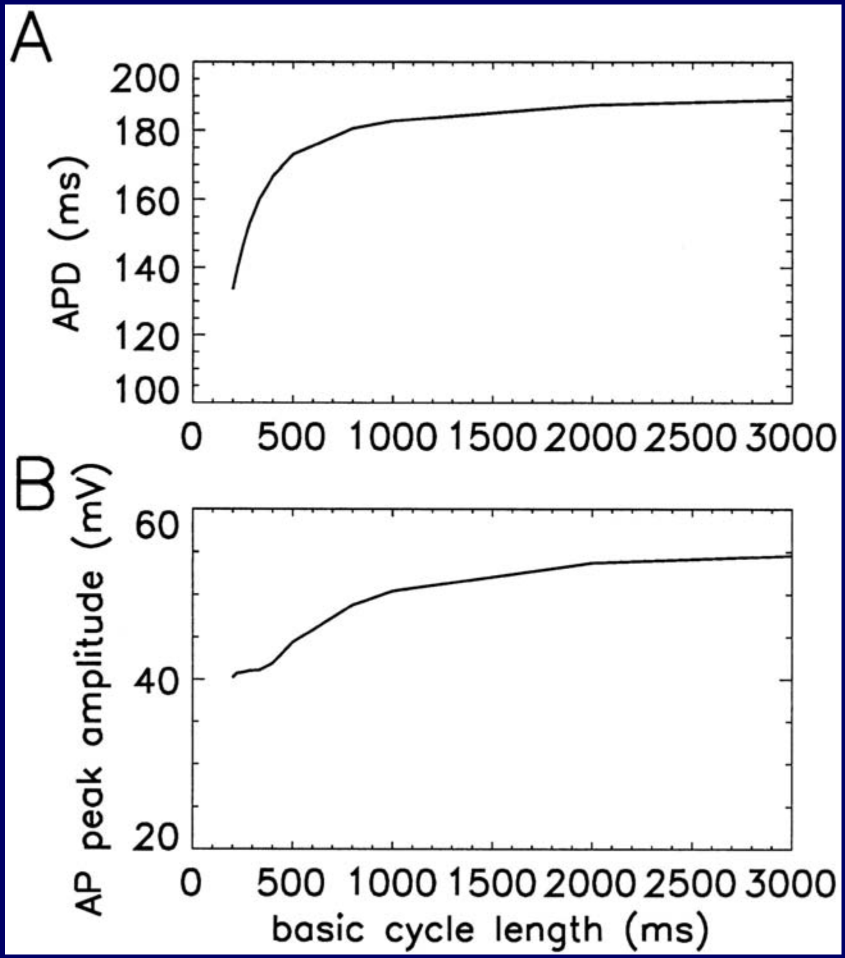
Force Frequency Relation

- Guinea pig, cat, and human have a domed shaped force-frequency relation
- Caused by the interplay of SR Ca Load and RyR adaptation.



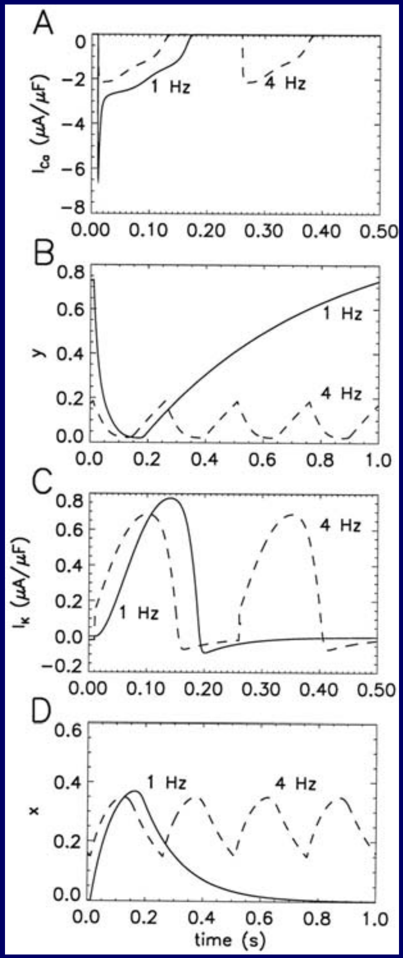
Action Potential Duration Restitution

- The model produces restitution of action potential duration.
- Previous studies by Rudy et al. suggested this was due to K^+ currents



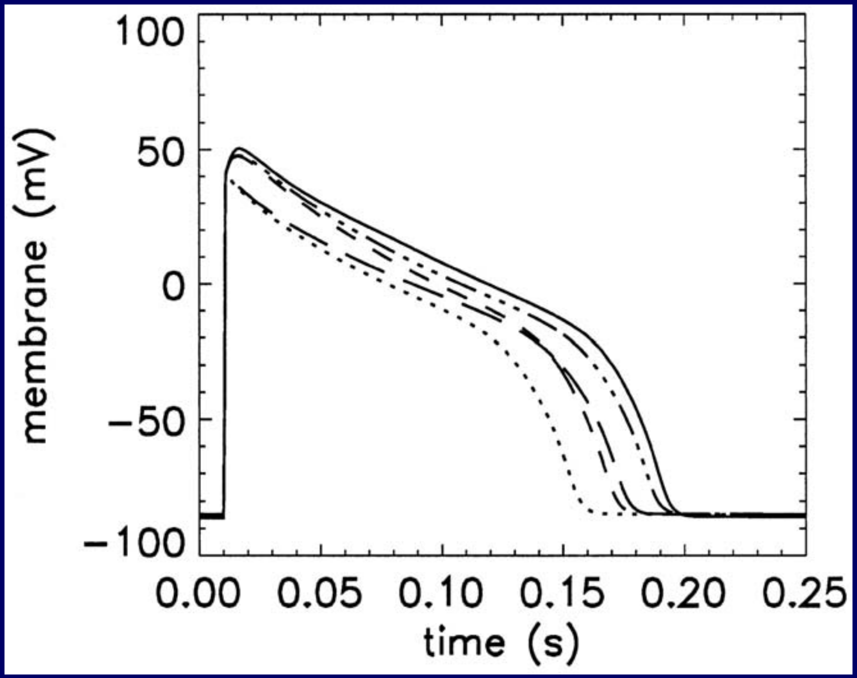
APD Restitution

- APD restitution is caused by both changes in the L-type current and potassium current (I_K) during pacing.

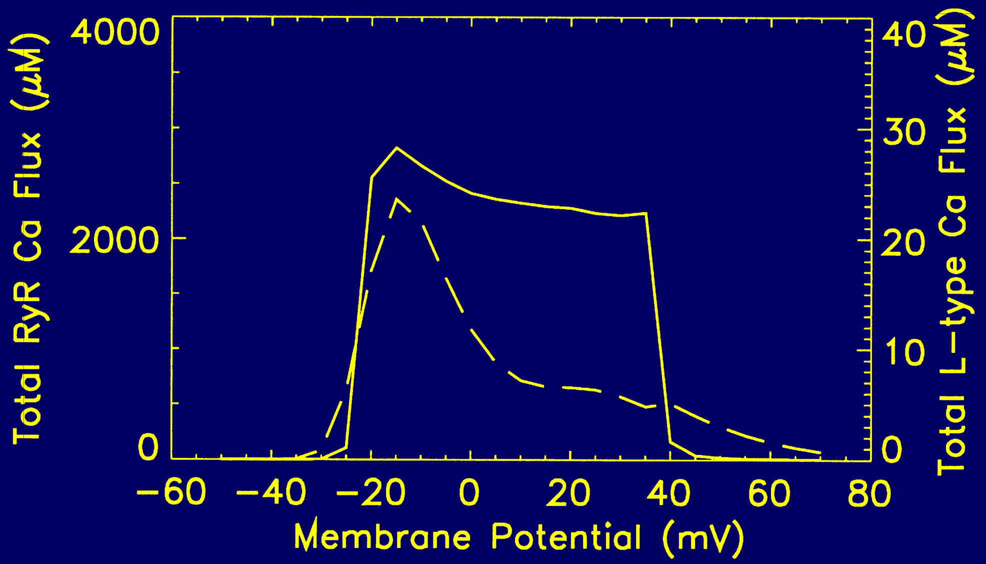


APD Restitution

- The contributions of various current to APD restitution.
- Dotted – 4 hz
- Dashed – L-type
- Long Dashed – I_K
- Dot-dashed – both
- Solid – 1 hz

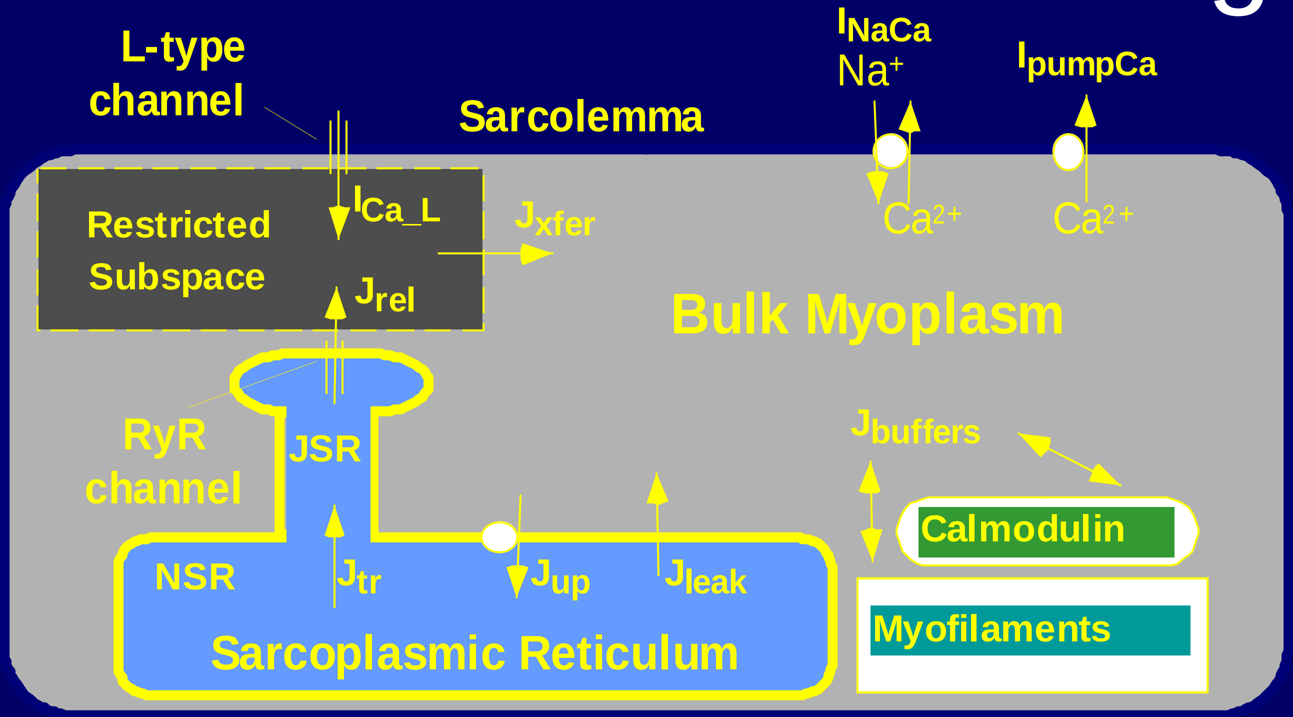


Jafri-Rice-Winslow Model for Cardiac Ca^{2+} Handling



- Common pool model
- Ca^{2+} cycling in cell
- Force-interval relations
- All or none

Jafri-Rice-Winslow model of Cardiac Ca^{2+} Handling

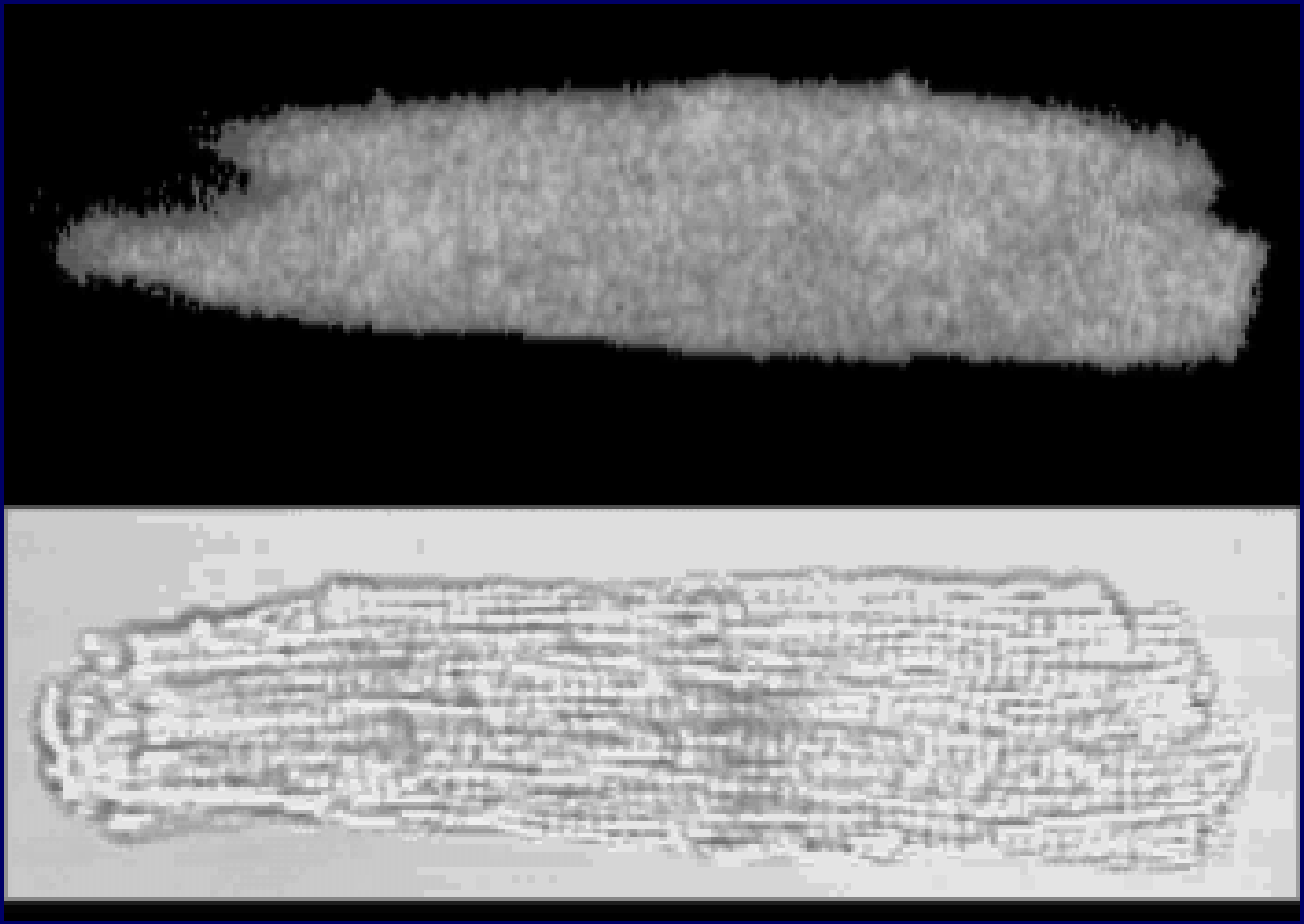


- Common pool model
- Ca^{2+} cycling in cell
- Force-interval relations
- All or none response

Presentation Overview

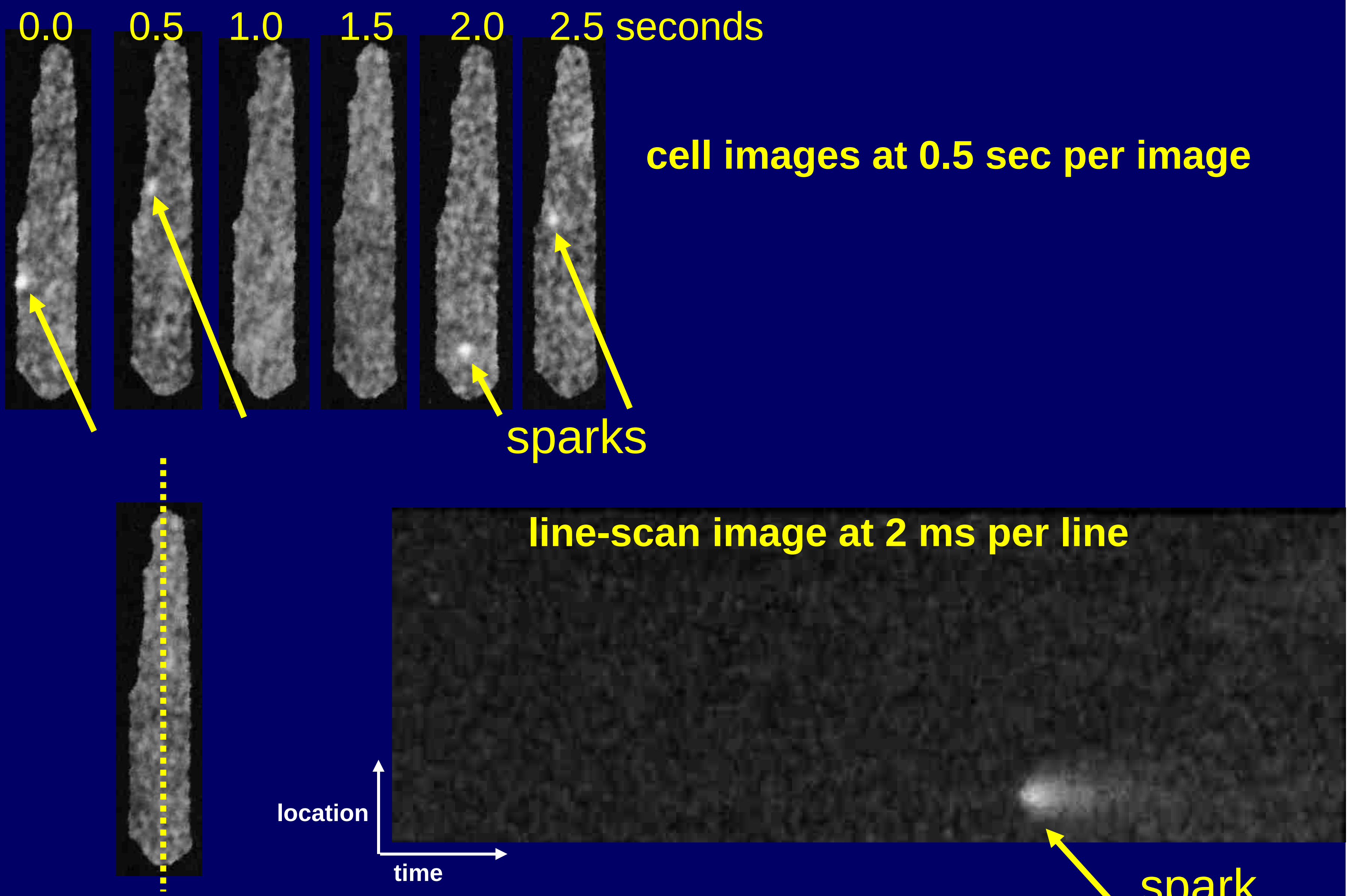
- Introduction
- Common Pool Models
- Calcium Sparks
  - What are the mechanisms of calcium release?
  - Can we explain experimental findings?
- Local Control
- Heart Failure

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)

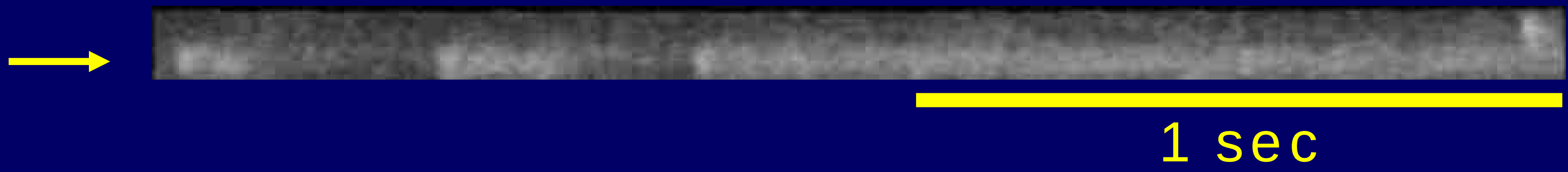
How do Ca^{2+} sparks terminate?

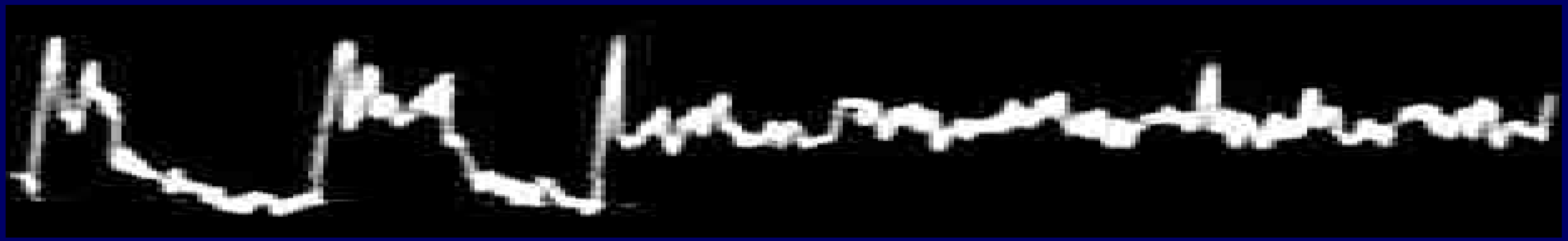
Three hypotheses have been proposed to explain the mechanism of Ca^{2+} spark termination:

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)

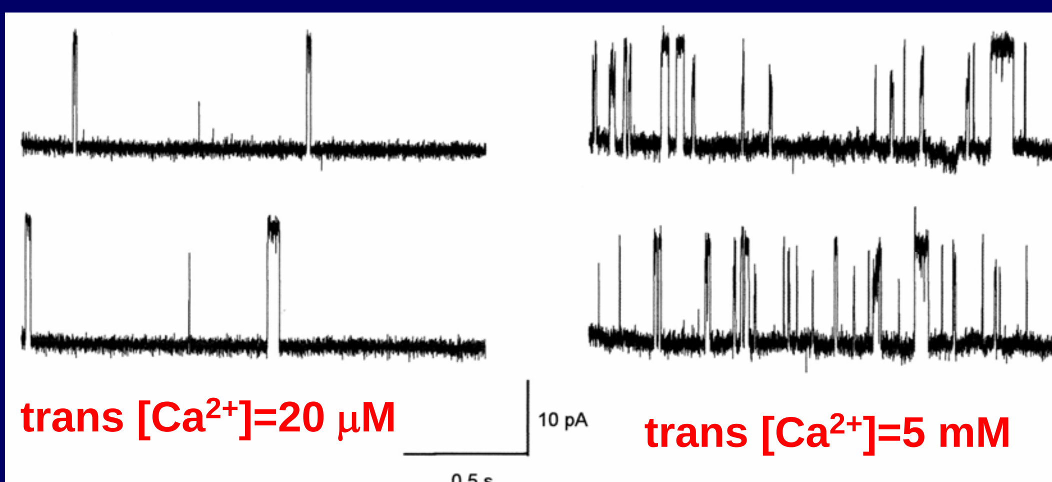
Recent experimental results suggested another hypothesis to us……

Hypothesis

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

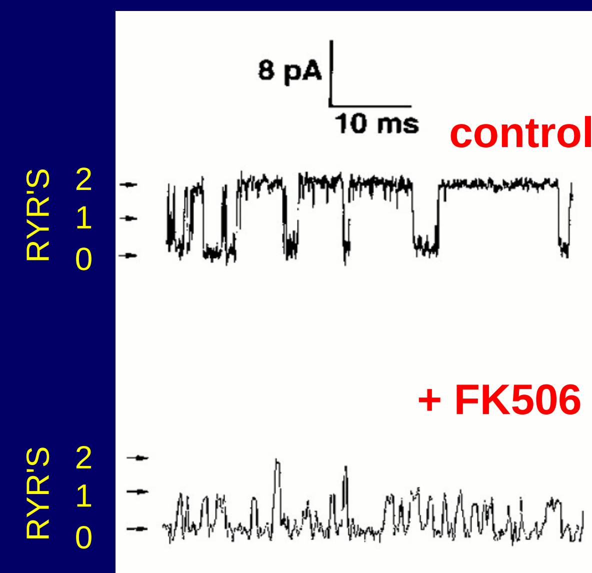
1. Large number of RyRs **(Franzini-Armstrong et al., 1998)**
2. SR lumenal $[Ca^{2+}]$
3. Coupled gating of RyRs

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



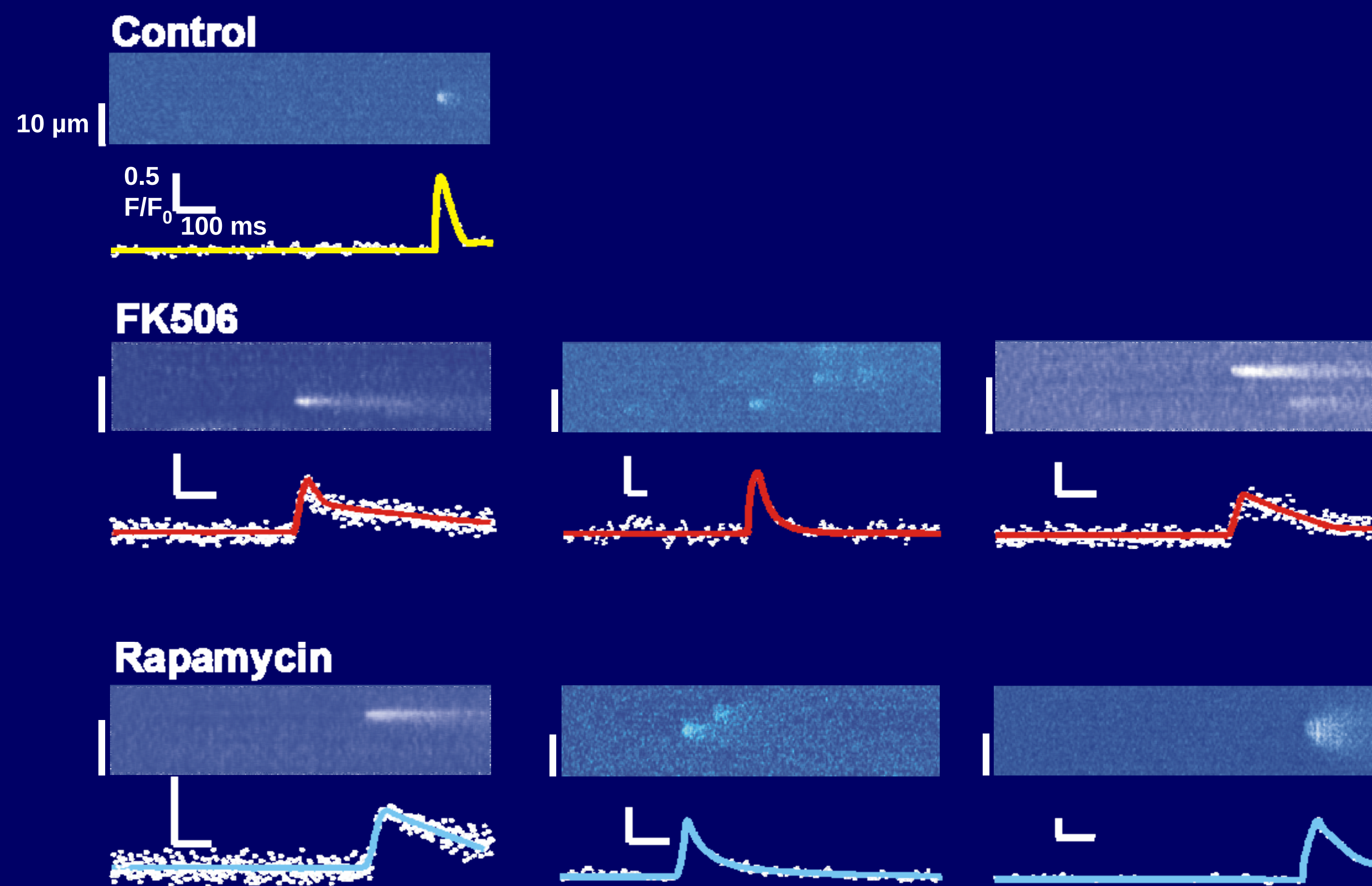
from Gyorke & Gyorke (1998) *Biophys J.* 75:280

Coupled gating of RyRs



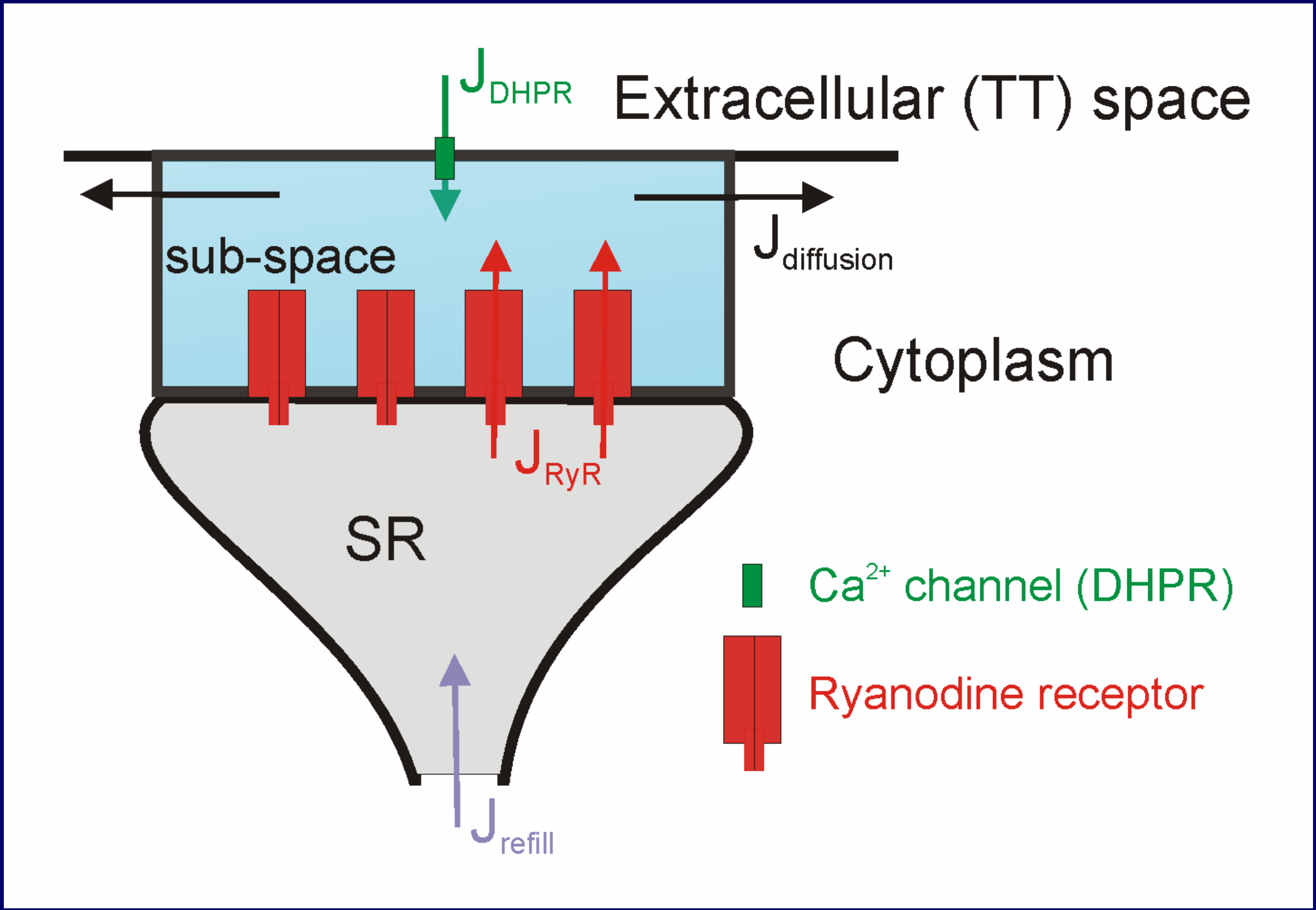
Skeletal Muscle RyRs: Marx et al., (1998) *Science* 281:818.
Heart RyRs: Gaburjakova et al. (2001) *Biophys. J.* 80:380A.

Experimental Results



Model: “Sticky Cluster”

Spatial organization



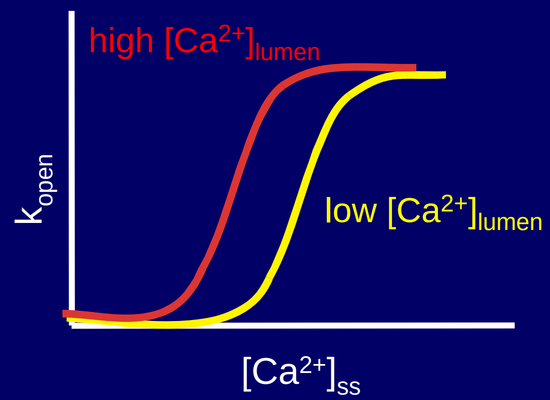
Model: “Sticky Cluster”

RyR Gating

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

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

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



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

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

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

Model Equations

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

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

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

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

where

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

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

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

where

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

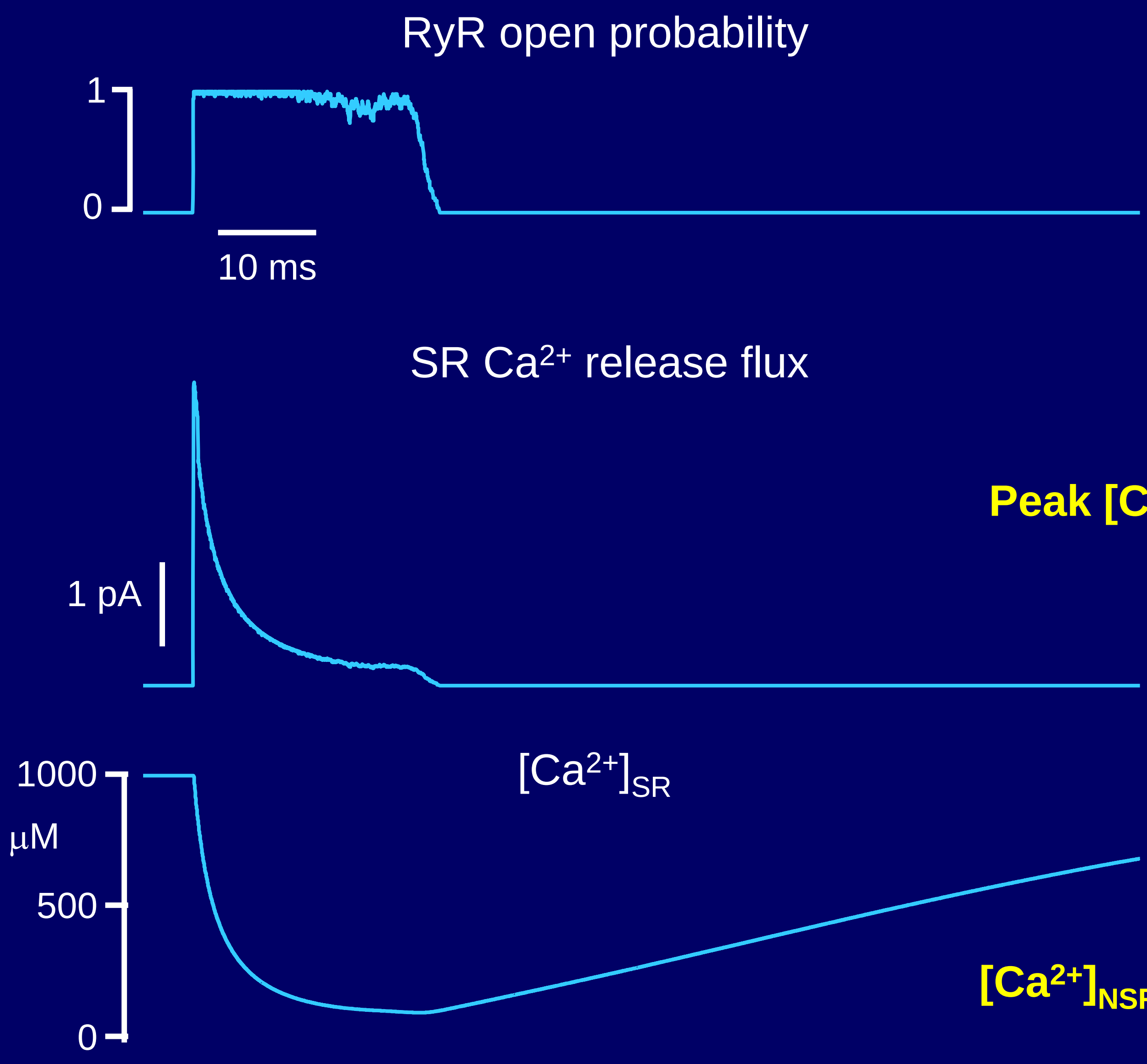
and

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

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 (40 ms) 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

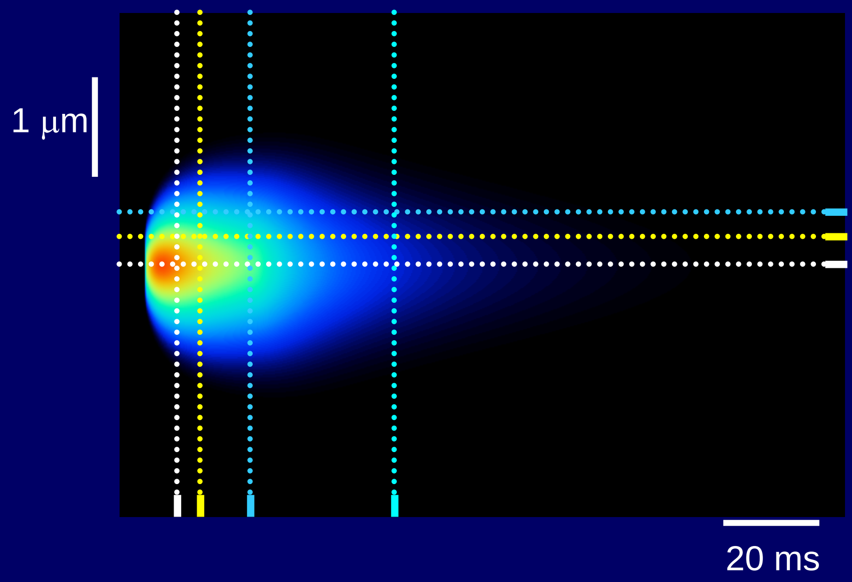


Peak $[Ca^{2+}]_{SS}$=~150 µM

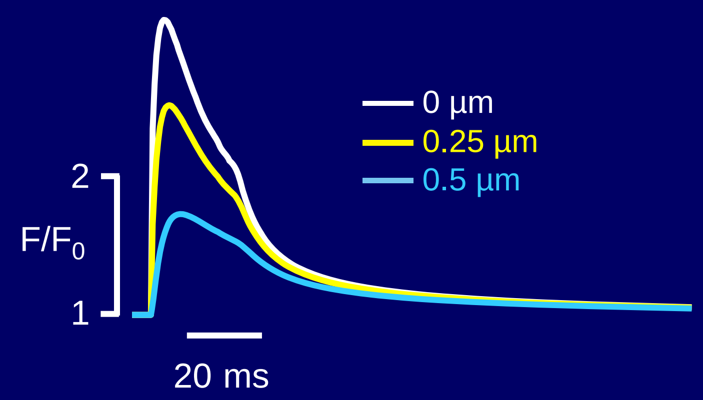
$[Ca^{2+}]_{NSR}$=1000 µM

Simulated Ca^{2+} sparks: control conditions

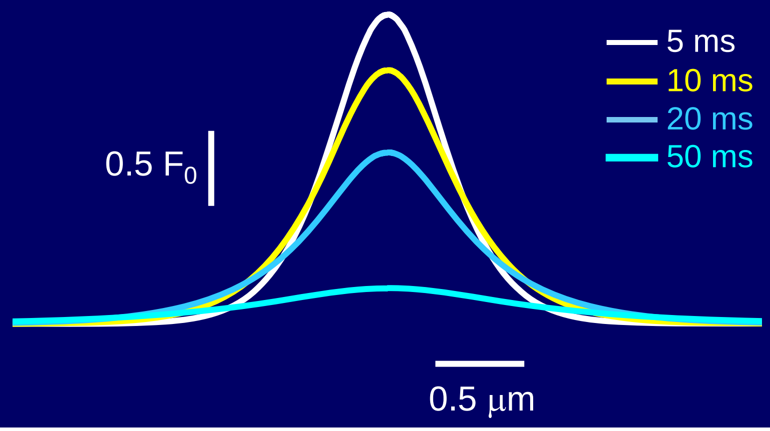
Ca^{2+} spark image



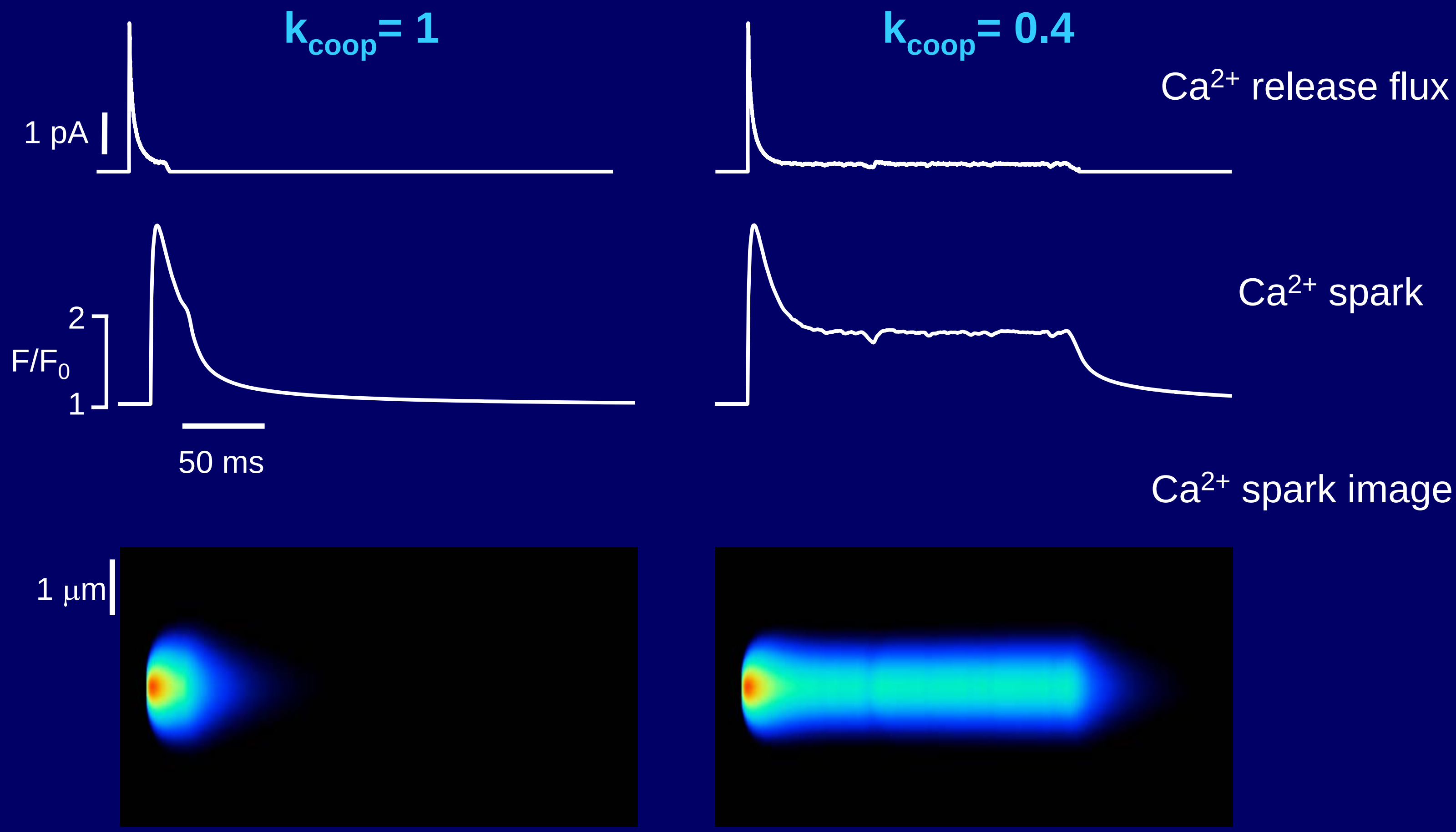
Ca^{2+} spark time course



Ca^{2+} spark spatial profiles

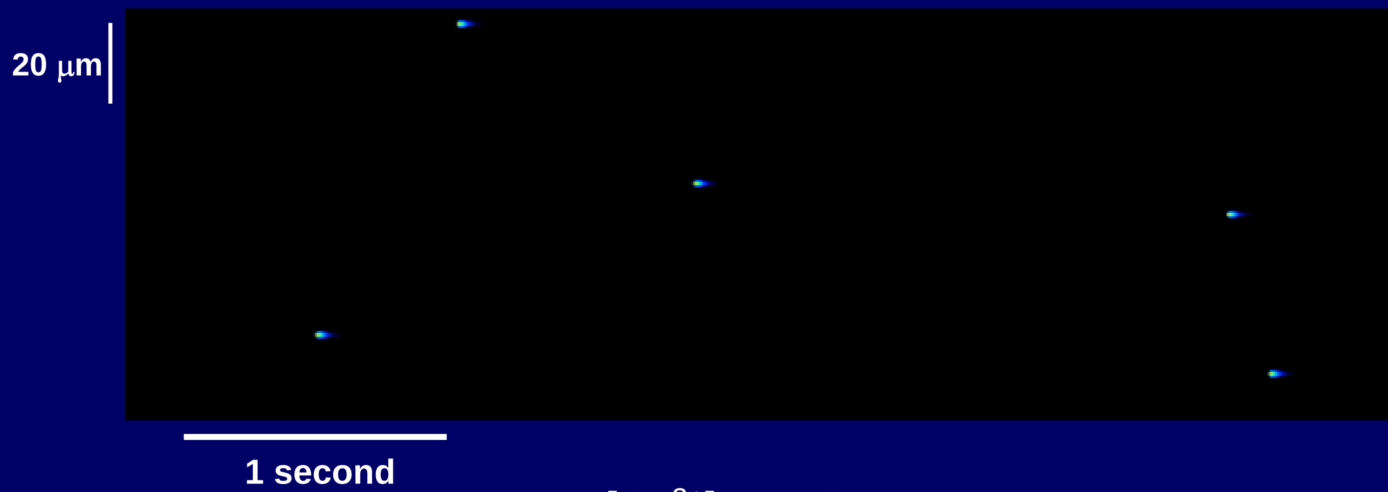


Simulated Ca^{2+} sparks: reduced coupling

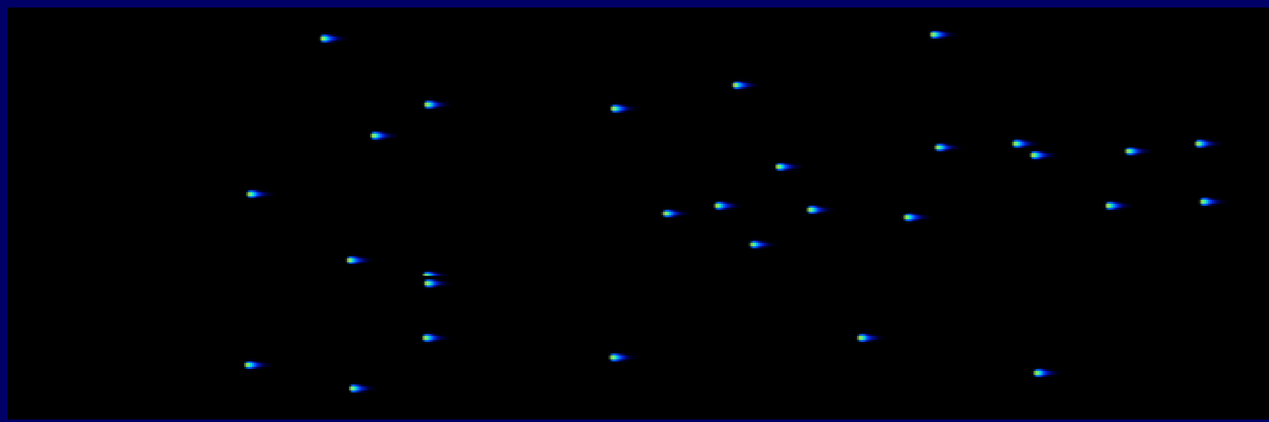


Spontaneous simulated Ca^{2+} sparks

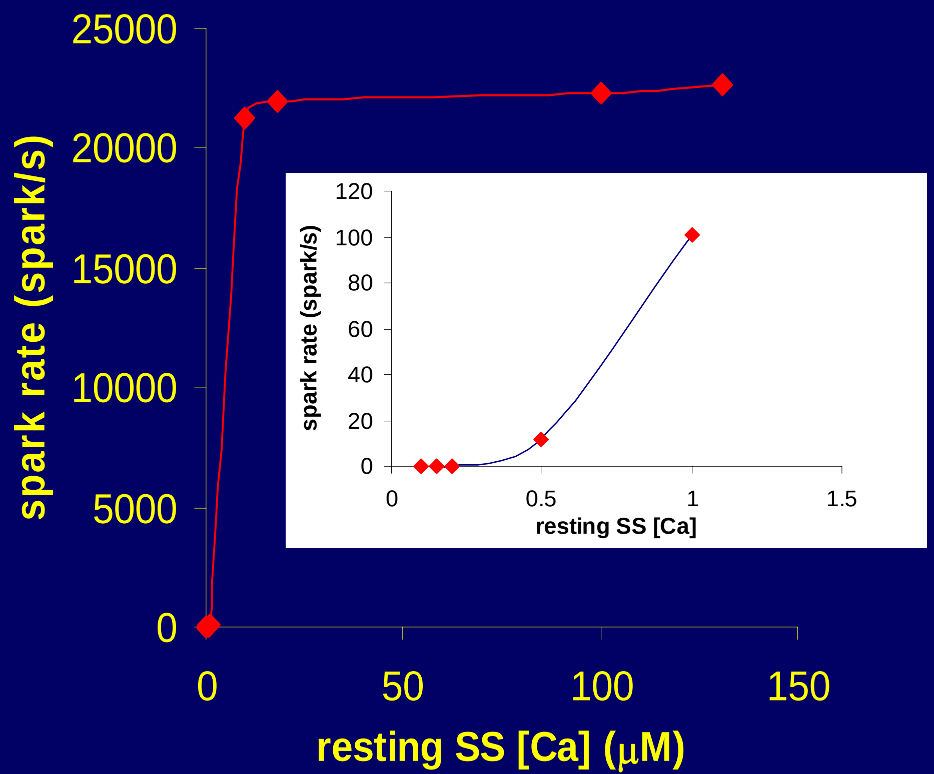
$[Ca^{2+}]_i$ = 100 nM

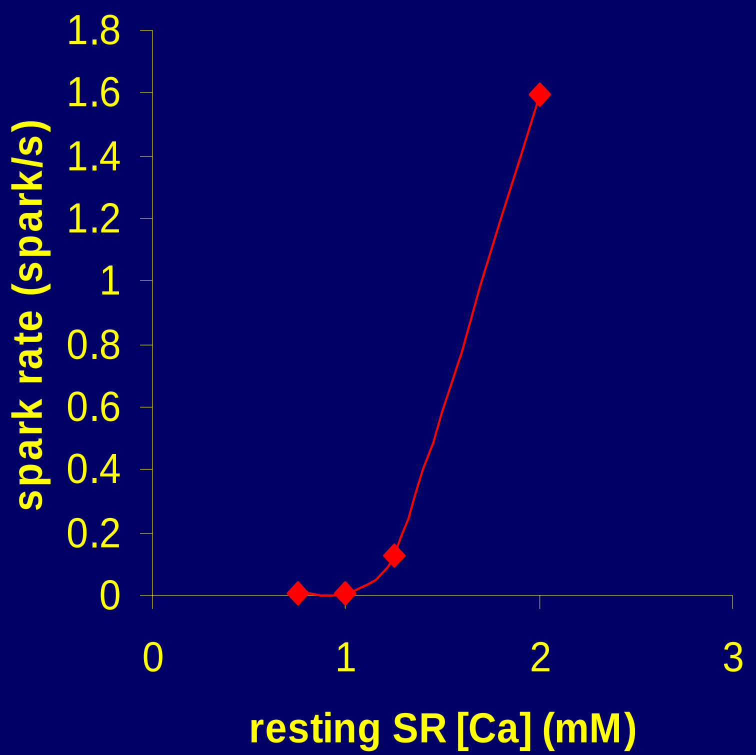


$[Ca^{2+}]_i$ = 150 nM

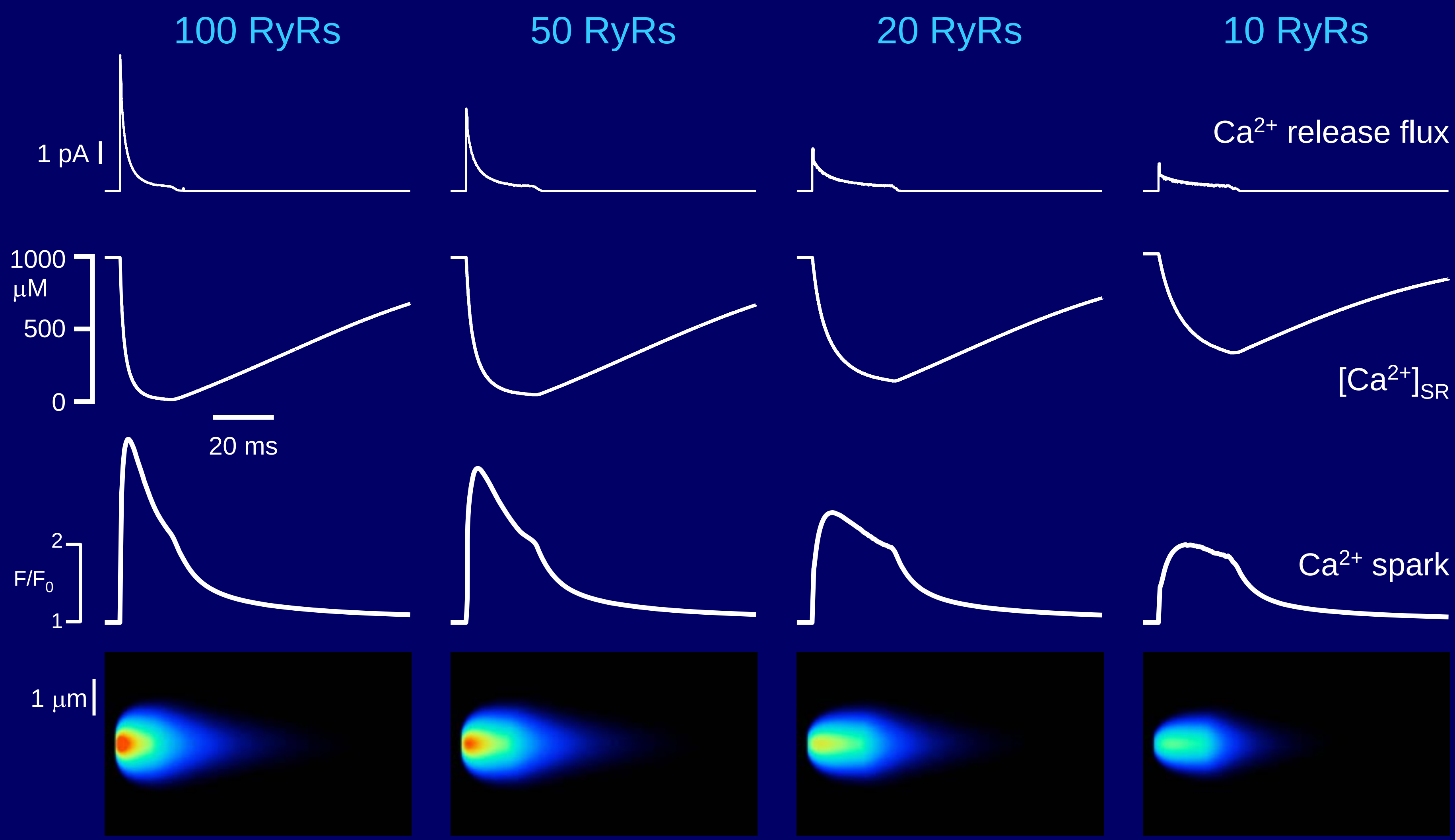


Spark Rate vs Subspace Ca^{2+} and SR Lumenal Ca^{2+}

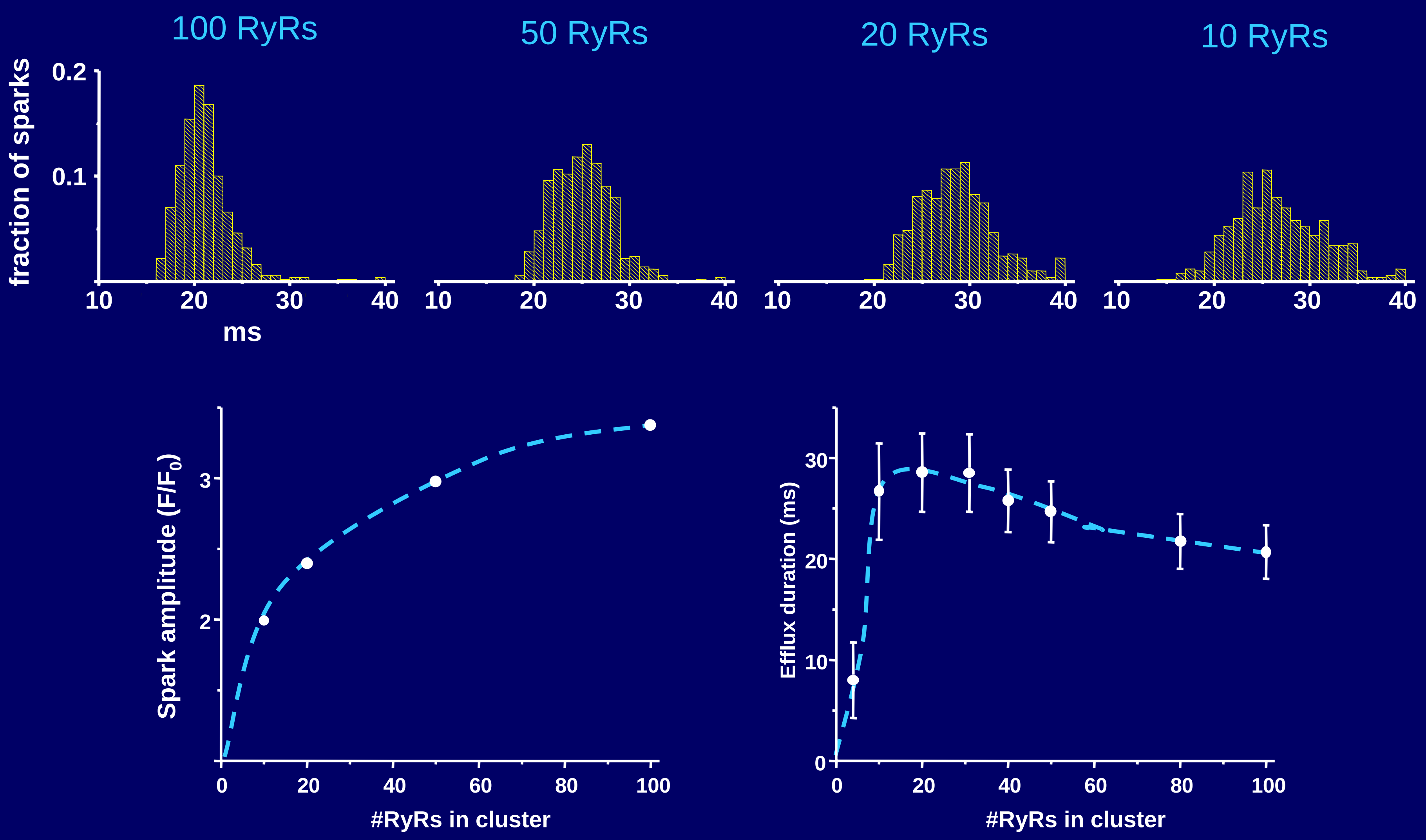




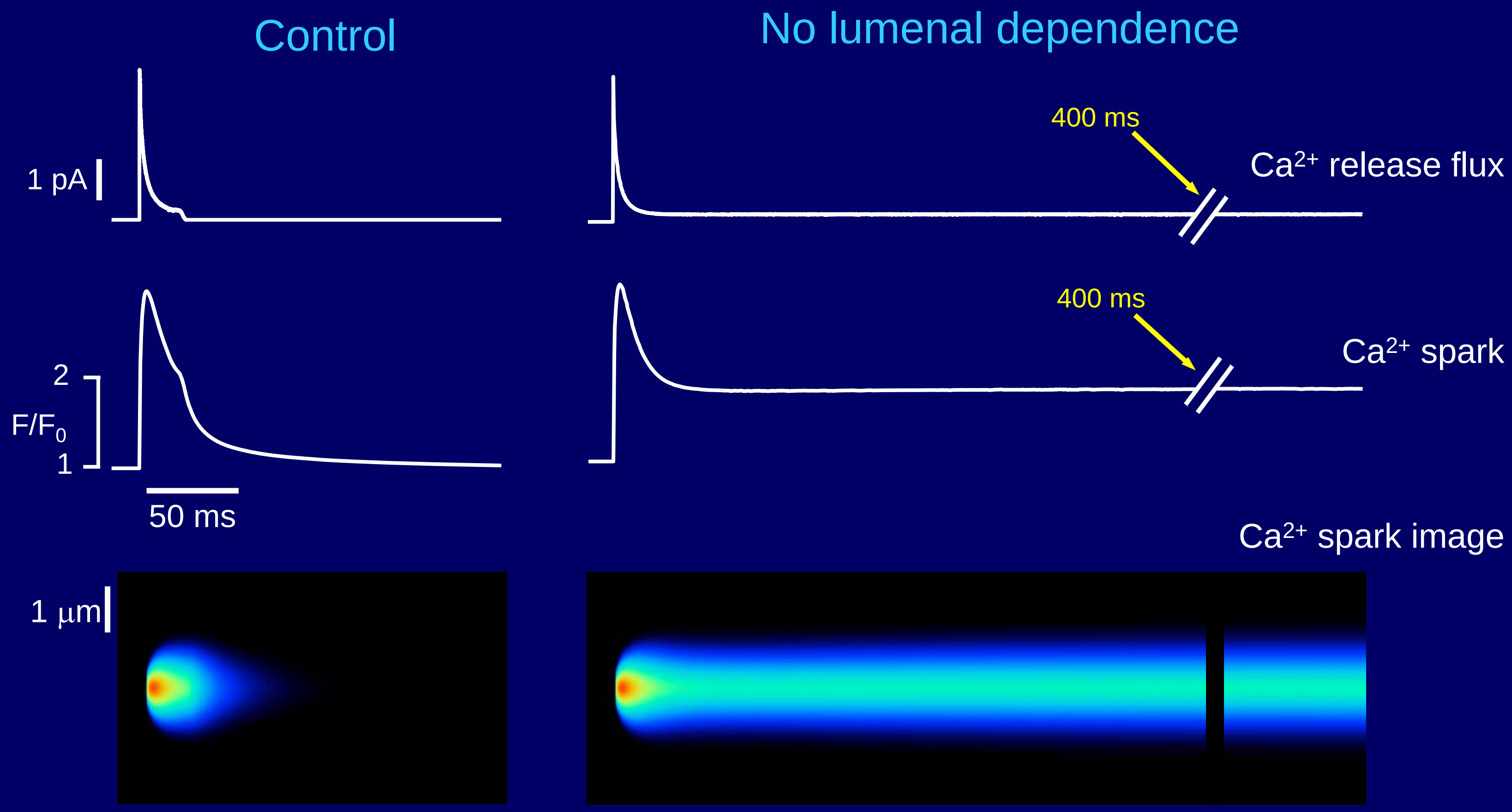
Simulated Ca^{2+} sparks: cluster size



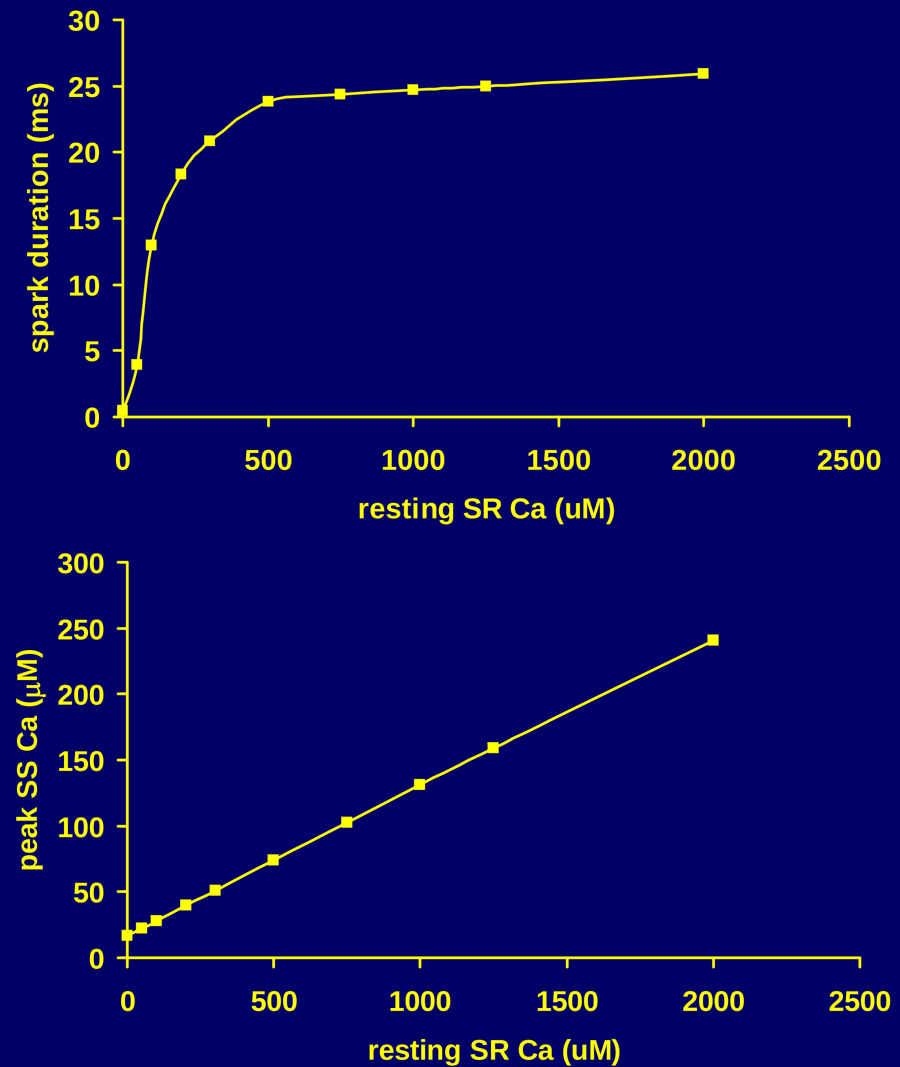
Simulated Ca^{2+} sparks: cluster size



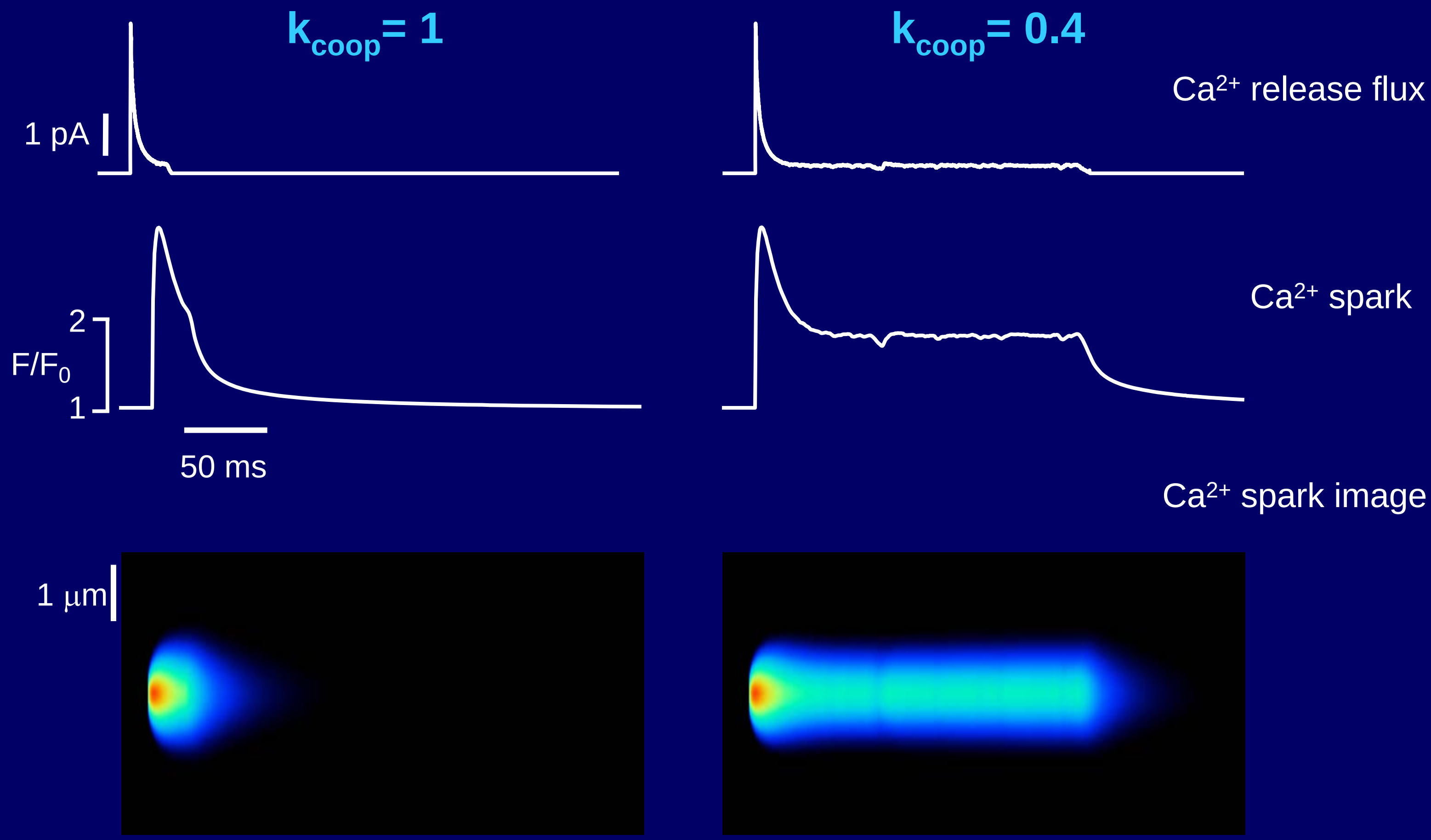
Simulated Ca^{2+} sparks: no lumenal dependence



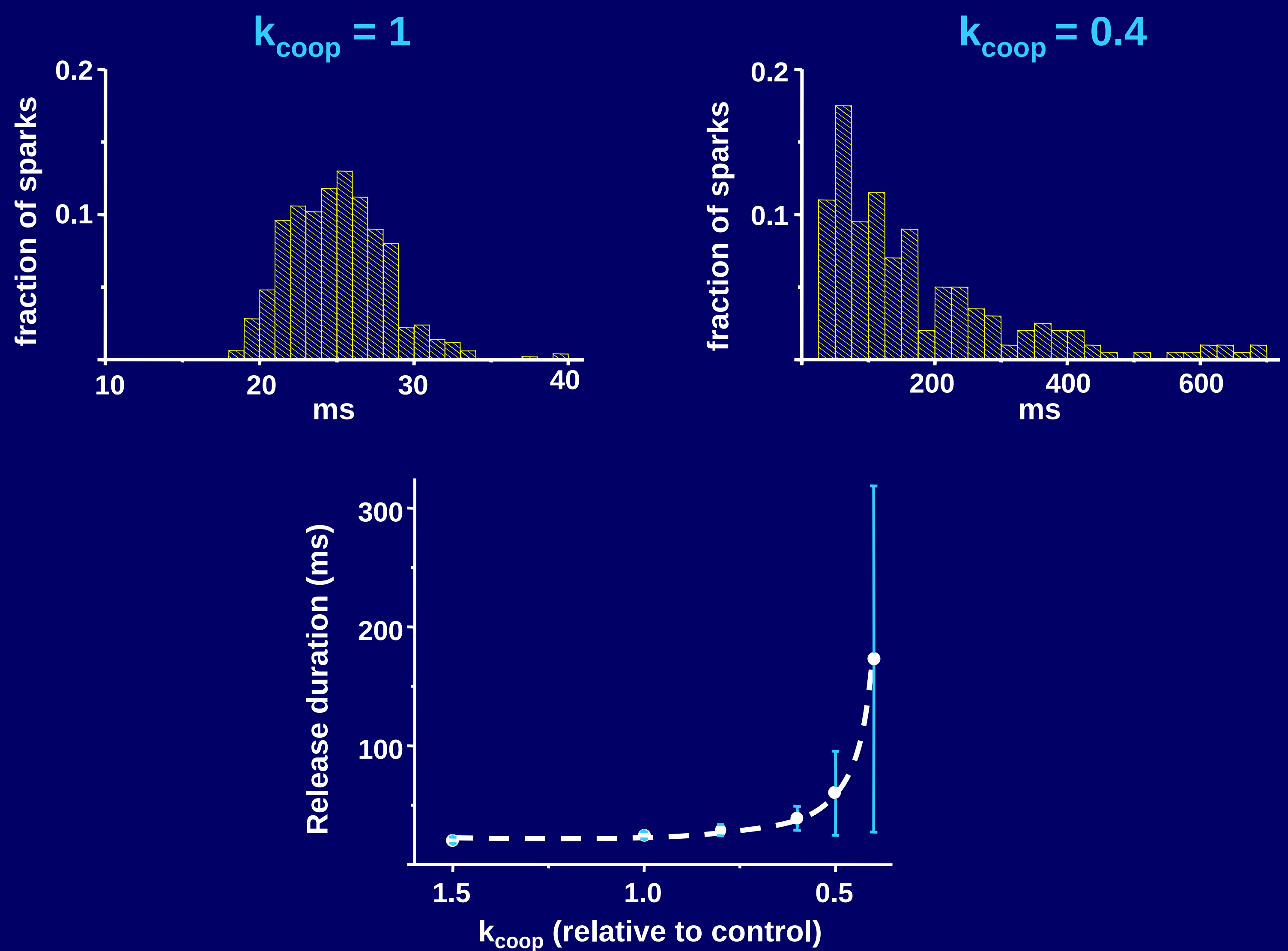
Simulated Ca^{2+} sparks: SR Load



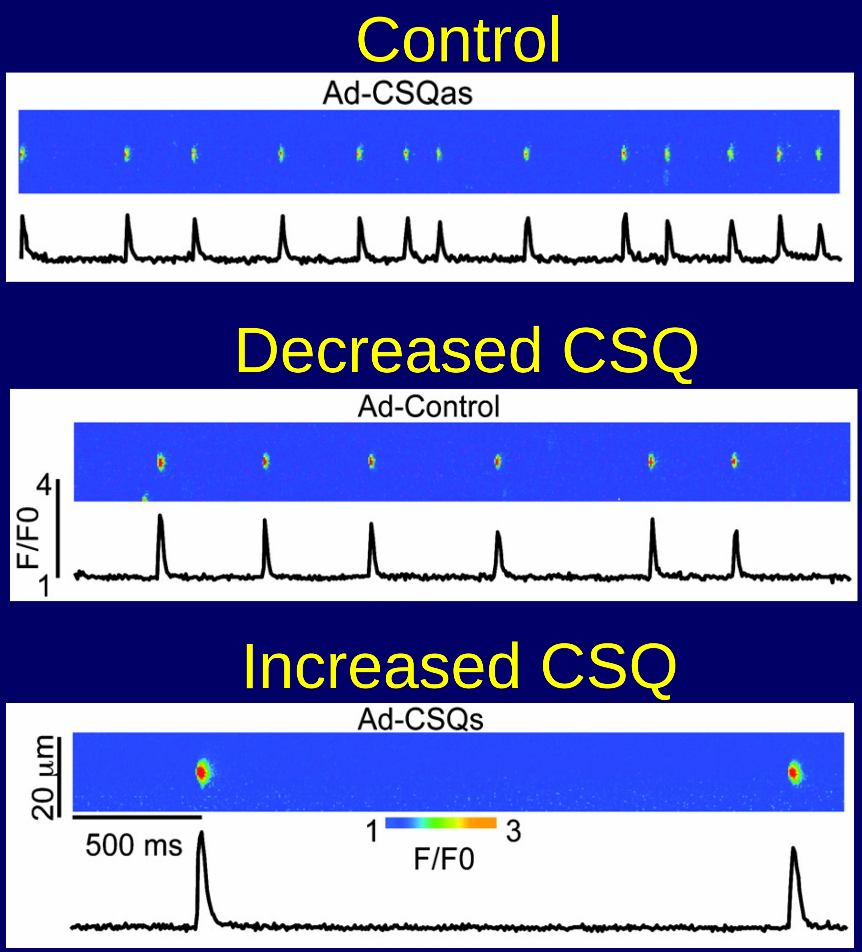
Simulated Ca^{2+} sparks: reduced coupling



Reduced coupling: population data



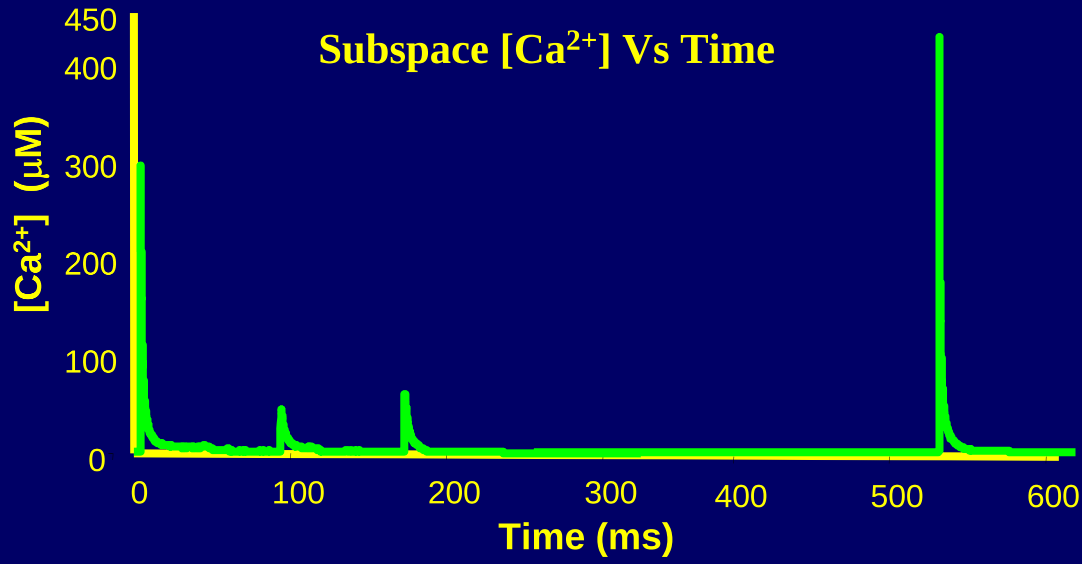
Calsequestrin and Sparks

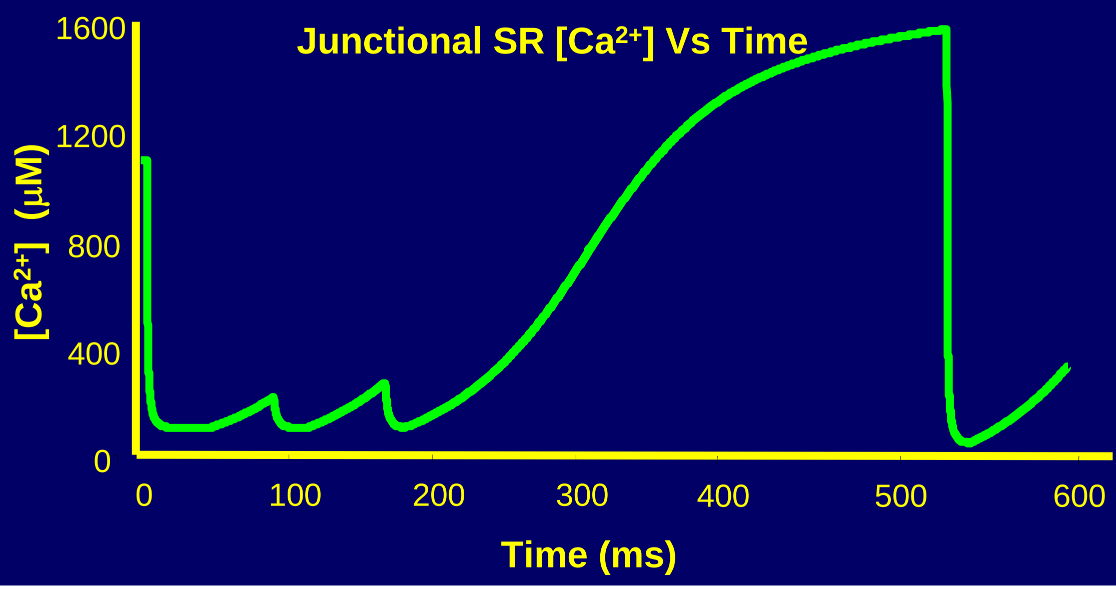


Terentyev et al., 2003

- 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.

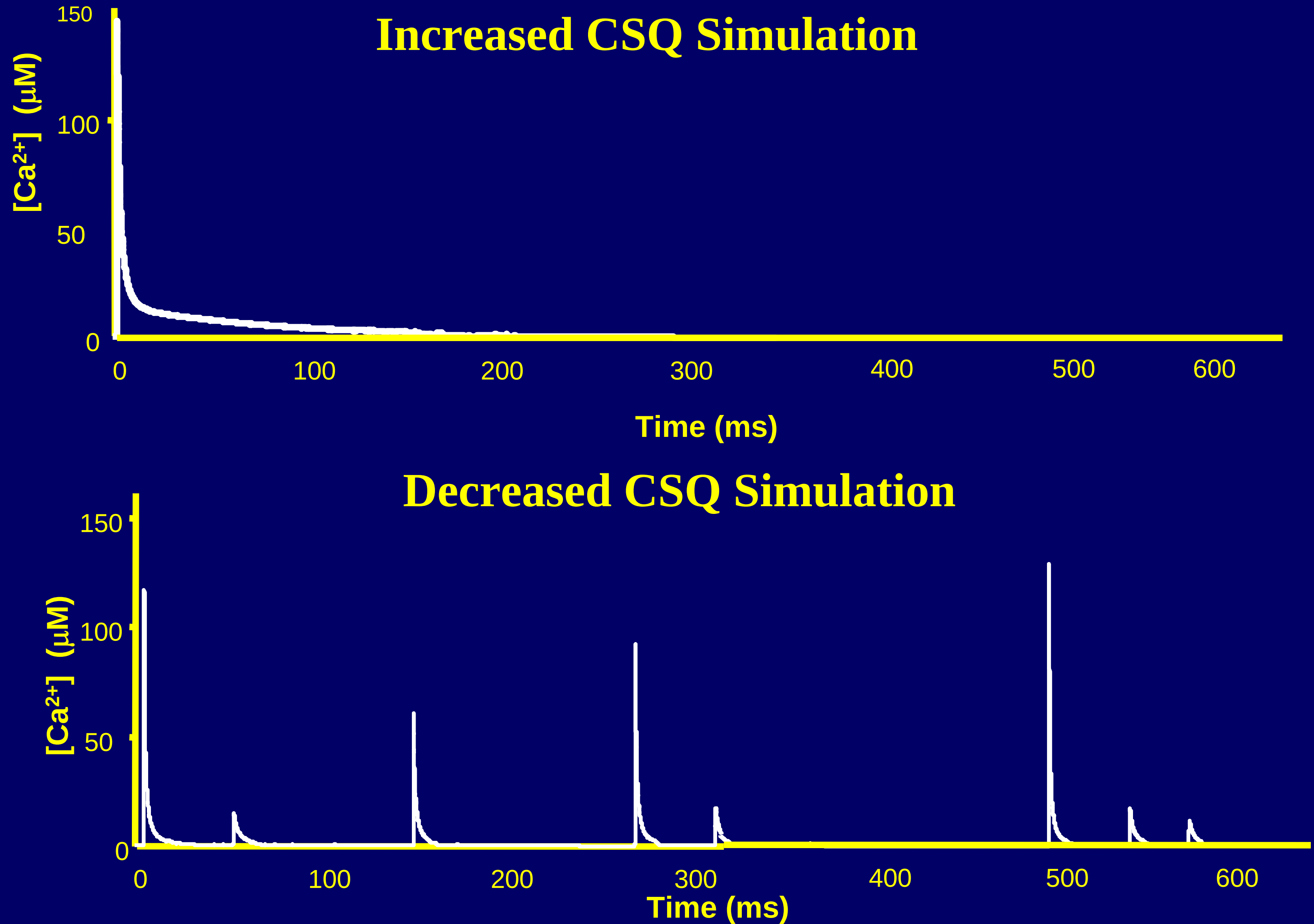
Spark Restitution





- 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

Ca^{2+} Sparks

- 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.

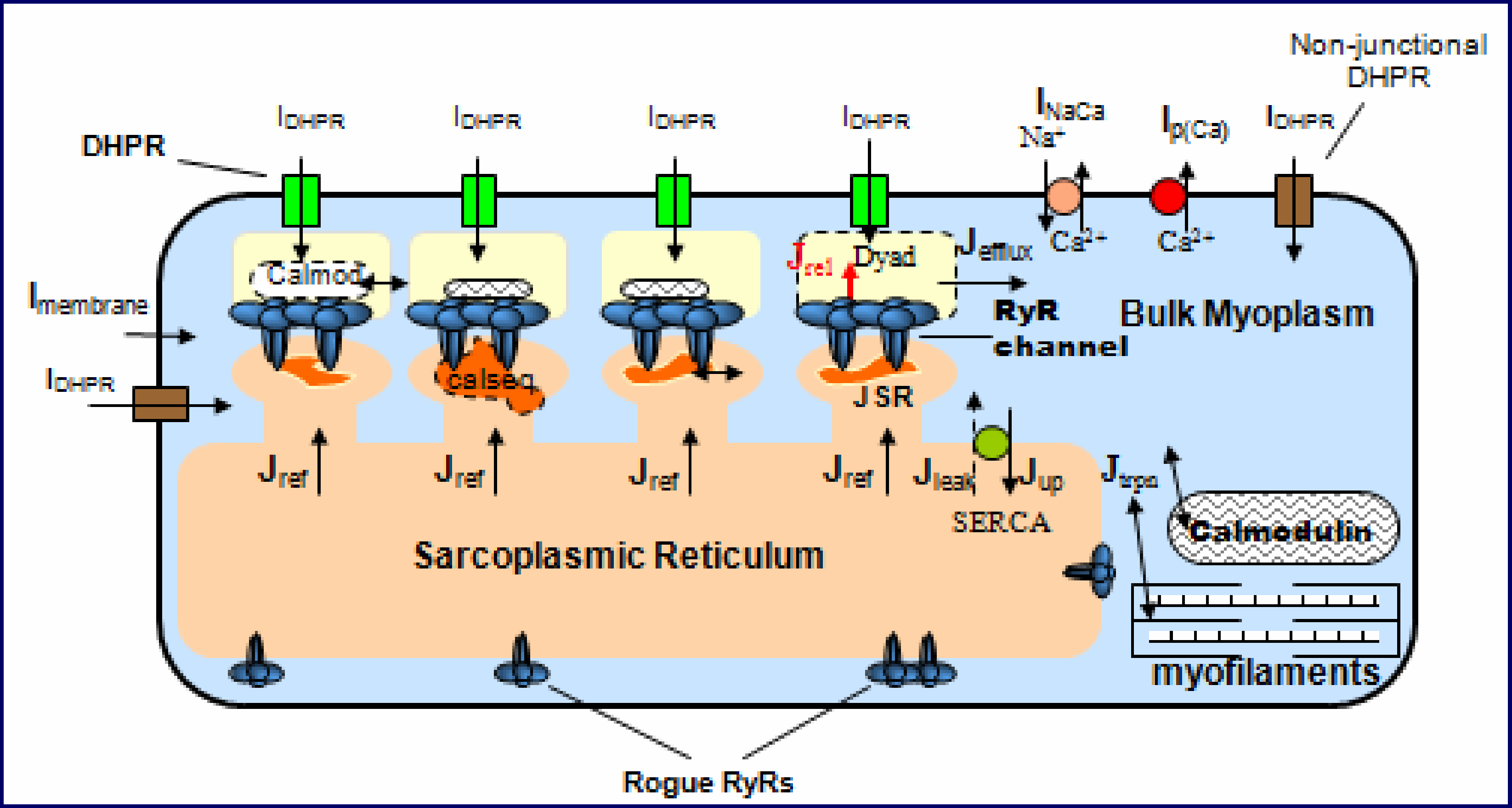
Presentation Overview

- Introduction
- Common Pool Models
- Calcium Sparks
- Local Control
  - Can we explain graded release?
  - Can we explain the sources of SR Ca Leak?
- Heart Failure

SR Leak

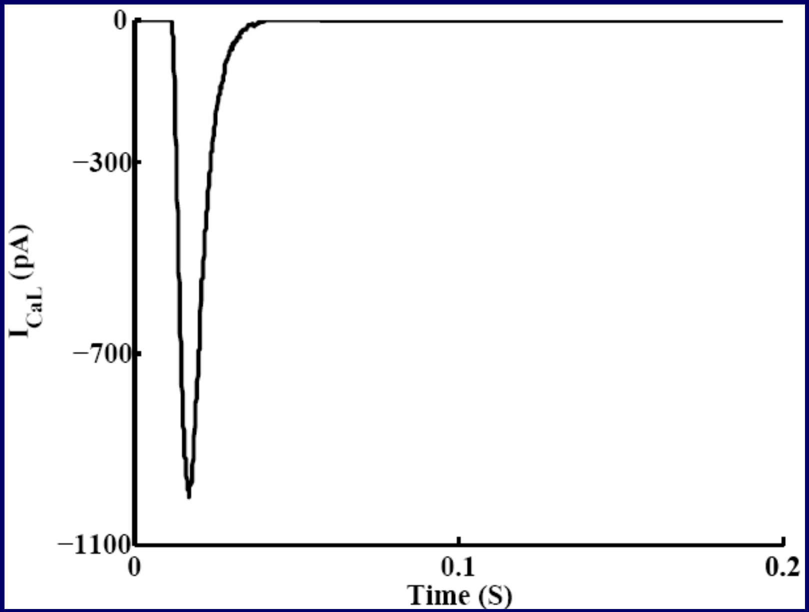
- The SR Ca^{2+} leak maintains homeostasis of SR Ca levels.
- Spontaneous Ca^{2+} sparks account for some but not all of the SR Ca^{2+} leak
- Addition of FK506 results in increase leak but no change in spark rate.
- Phosphorylation results in changes in leak but no change in spark rate.

Model Schematic

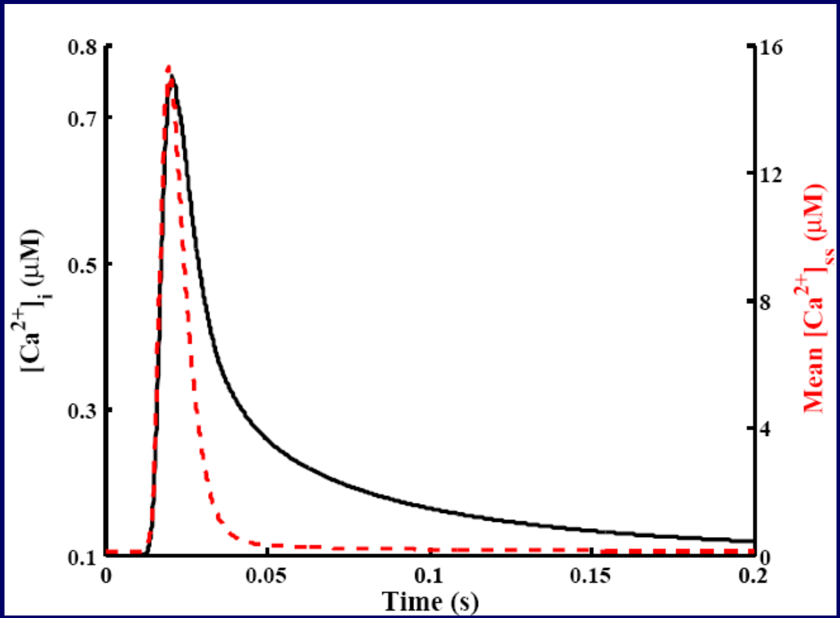


Ca^{2+} transients

Whole-cell Ca^{2+} current

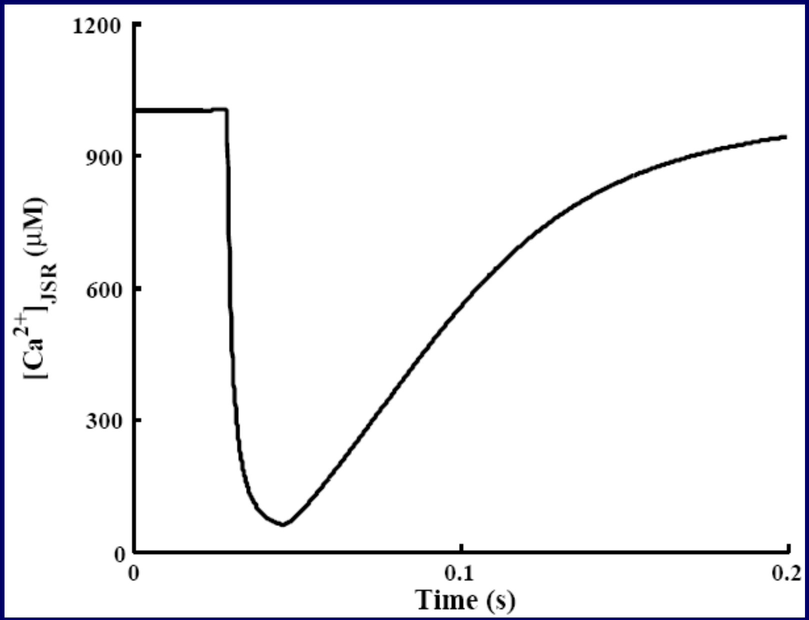


Cytosolic and mean subspace Ca^{2+} transients

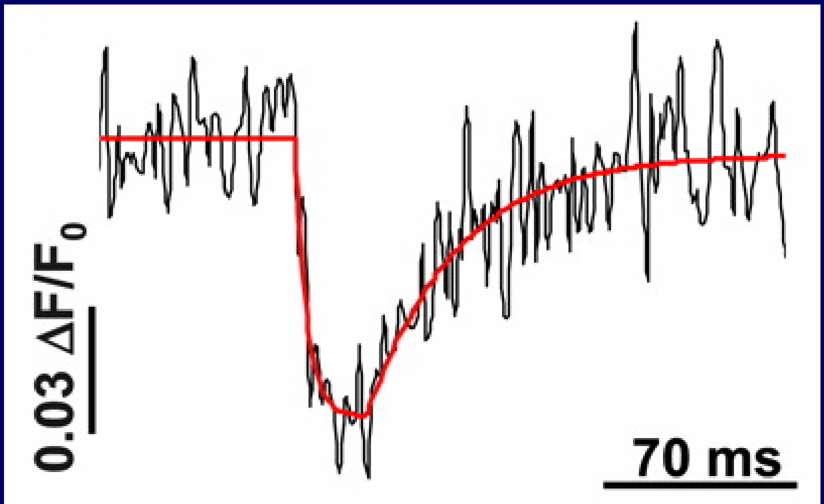


JSR Ca^{2+} Transient

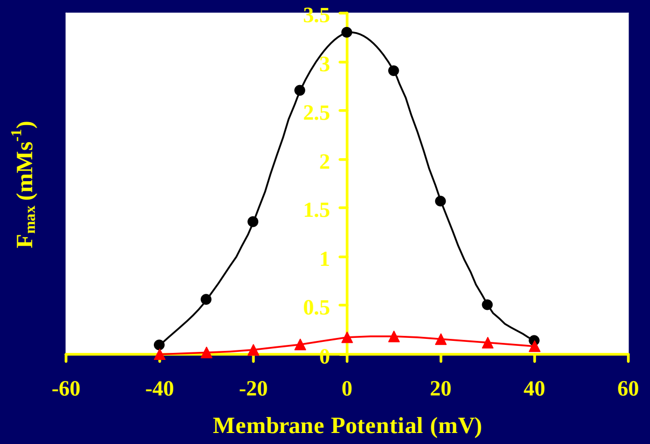
Sample Ca^{2+} levels in the JSR

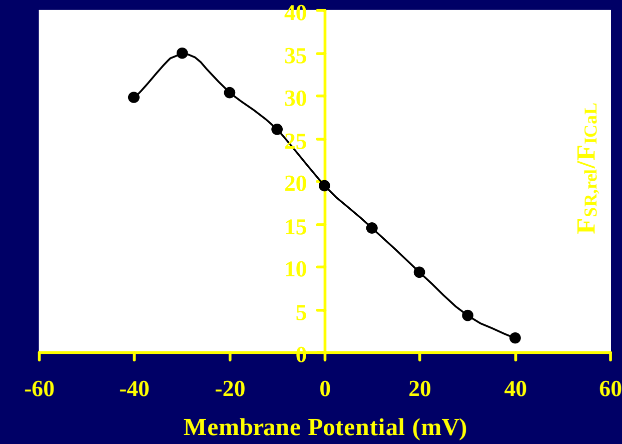


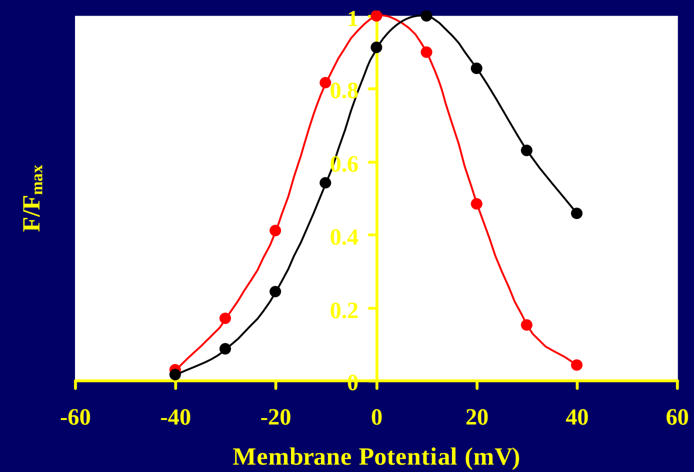
Brochet et al. 2005



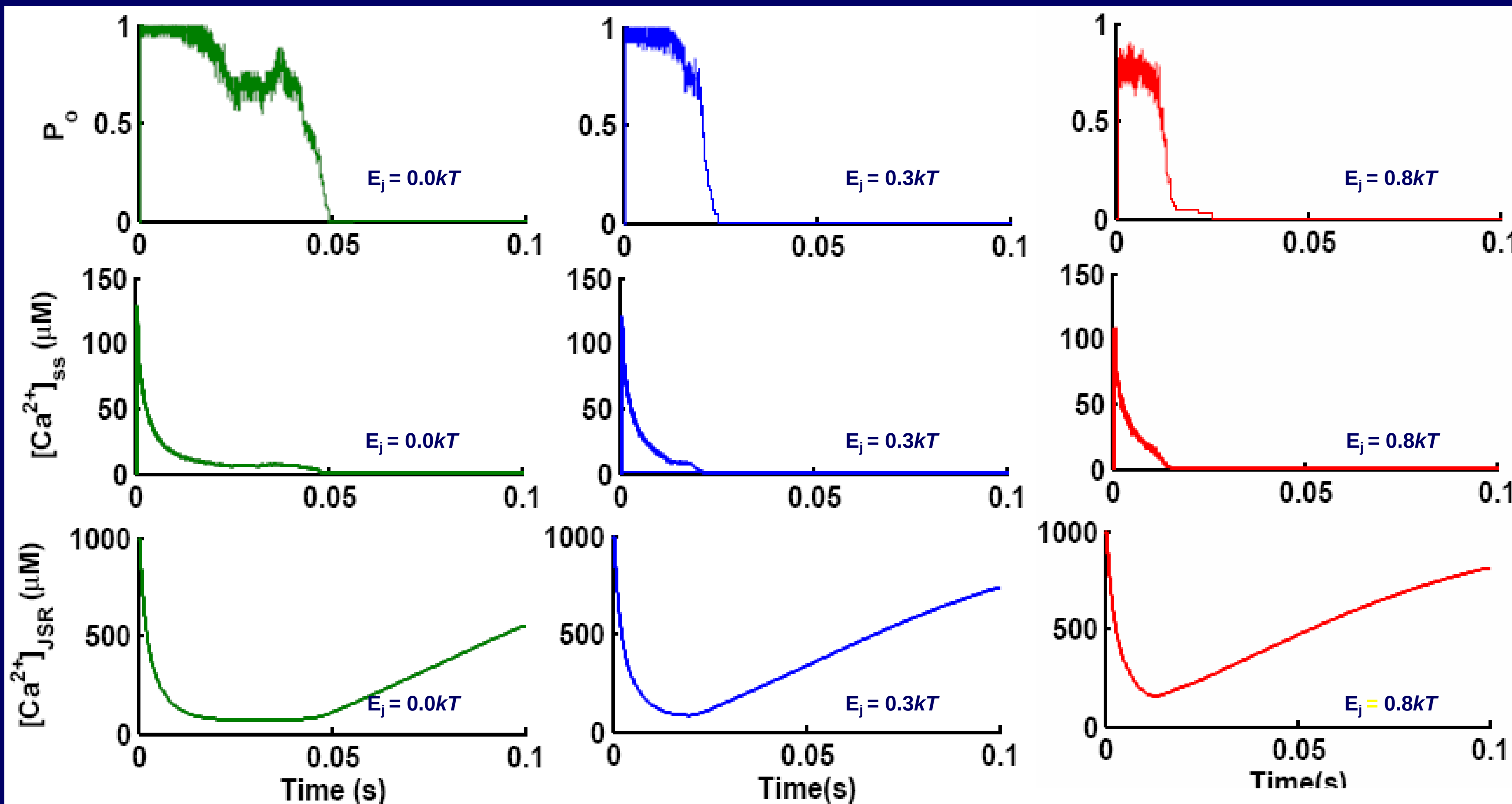
Graded Release in Whole Cell Model



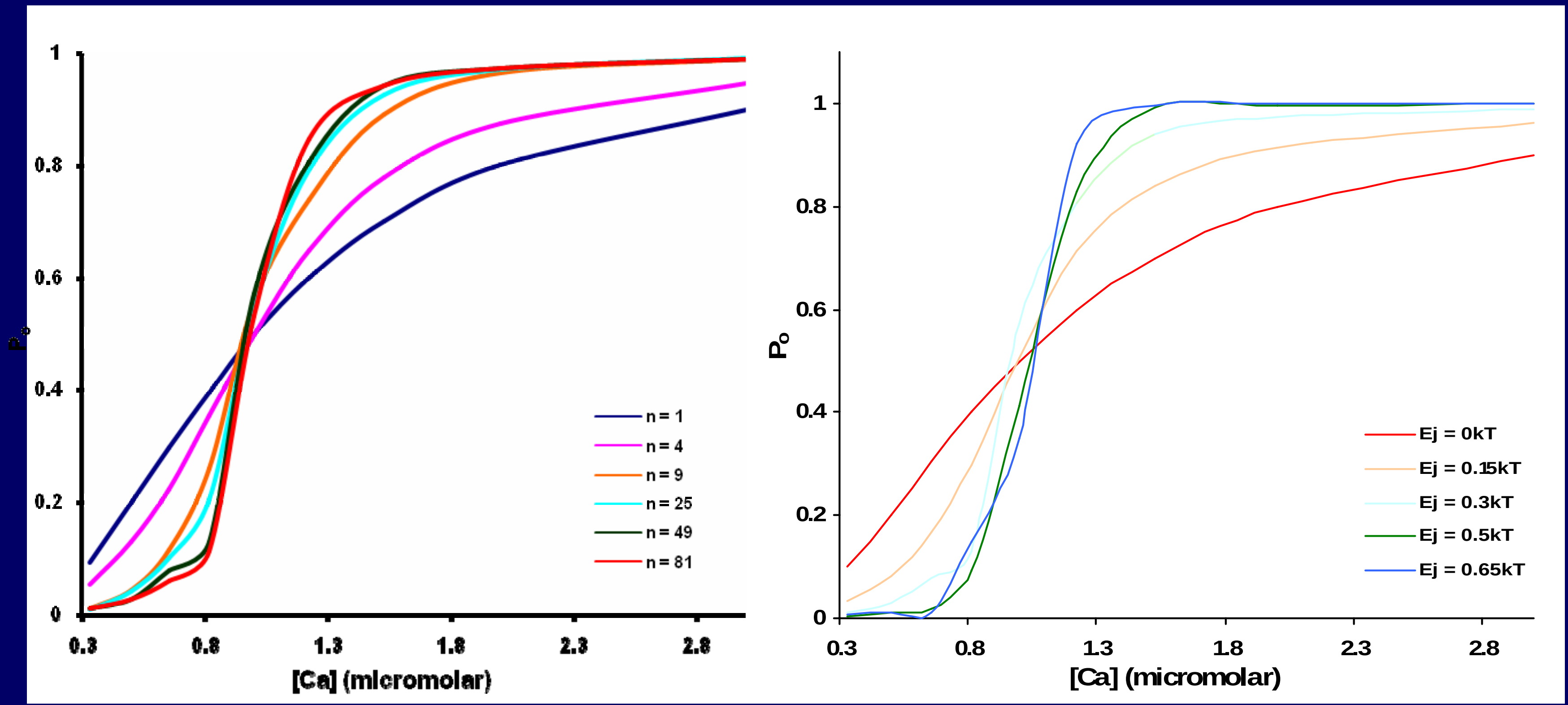




Coupling manipulation

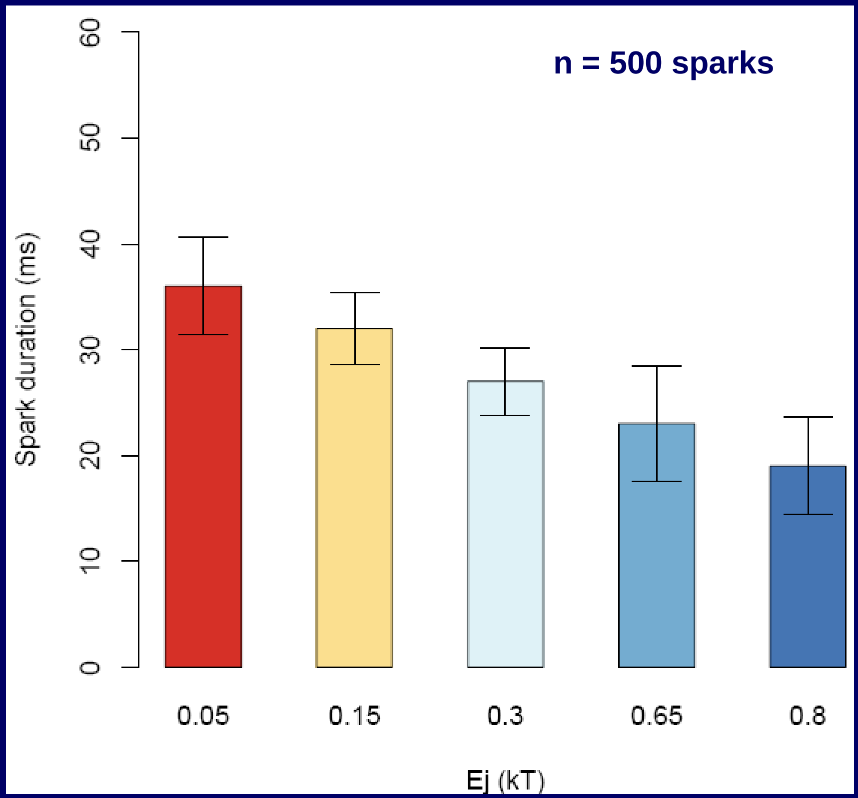


Coupling Manipulations

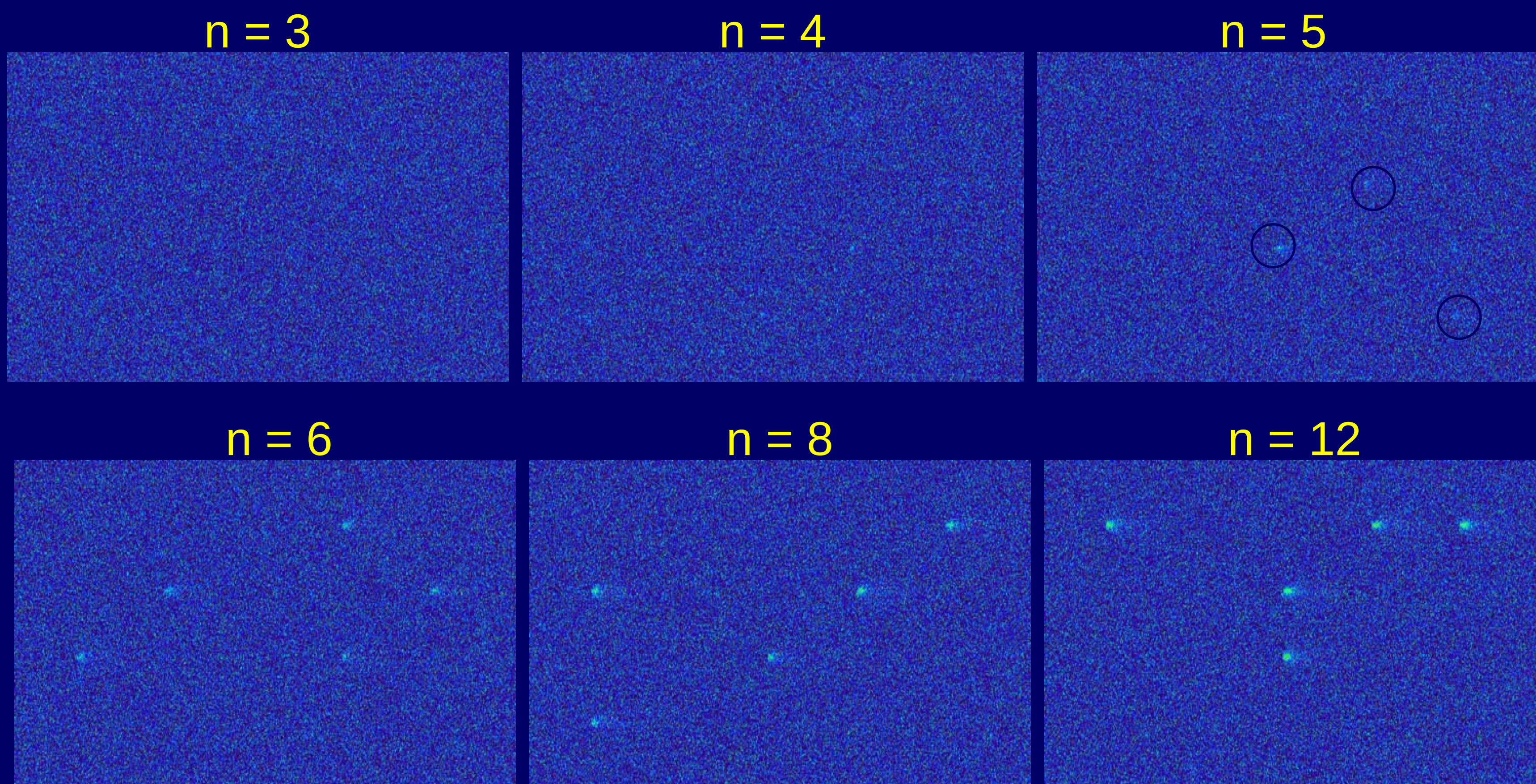


Coupling and Spark Duration

- Spark duration decreases with increased coupling
- This could explain why addition of FK506 decreases leak without affecting spark rate.

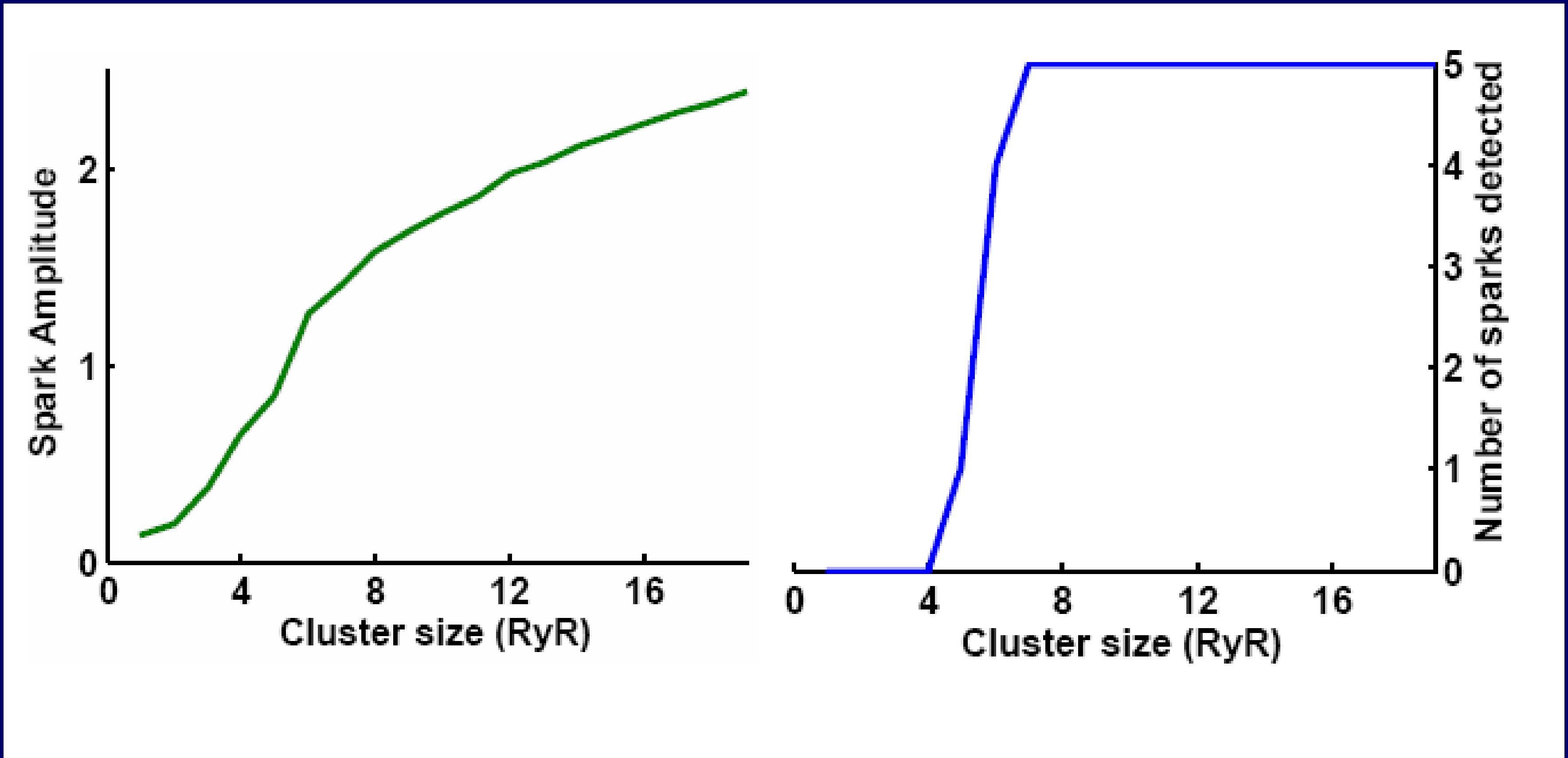


Imaging Sparks



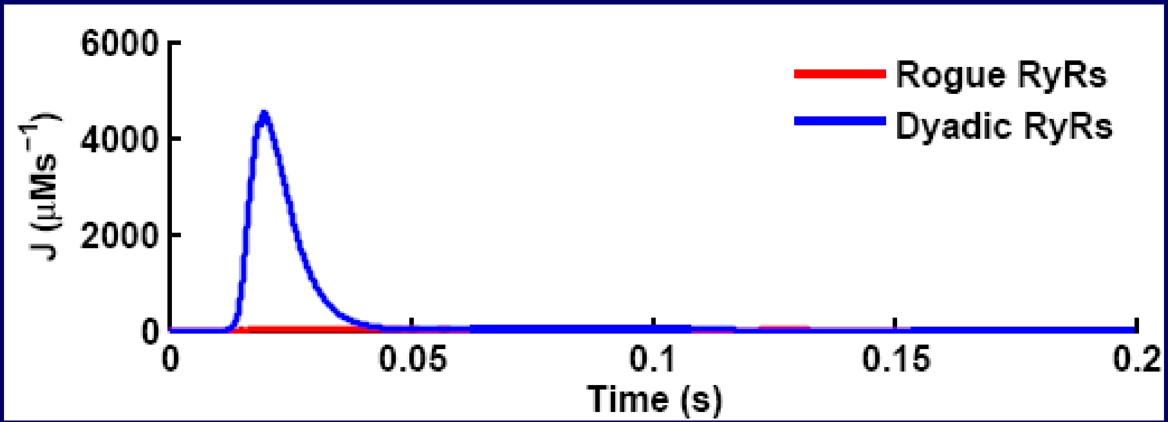
B **C**

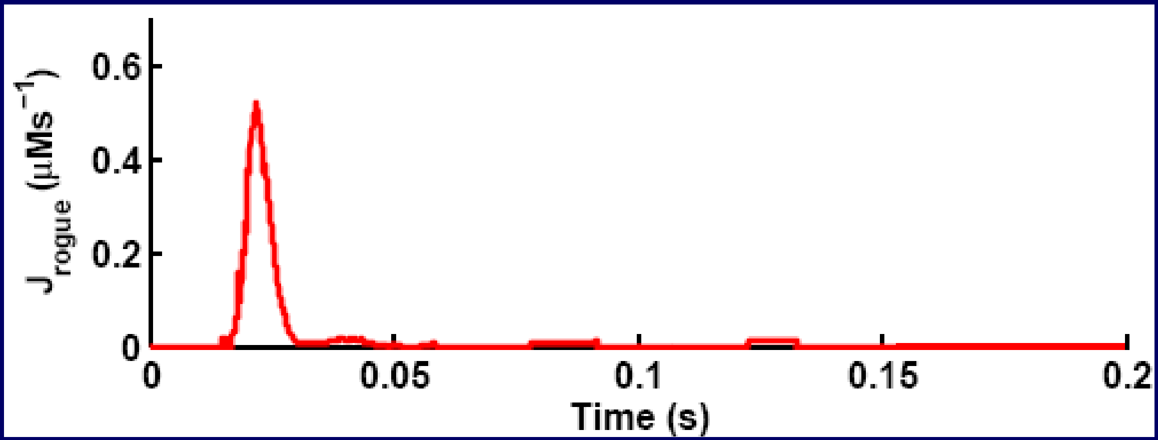
Spark Detection



Openings of only a few ryanodine receptors (<5) cannot be resolved experimentally

Systolic Leak

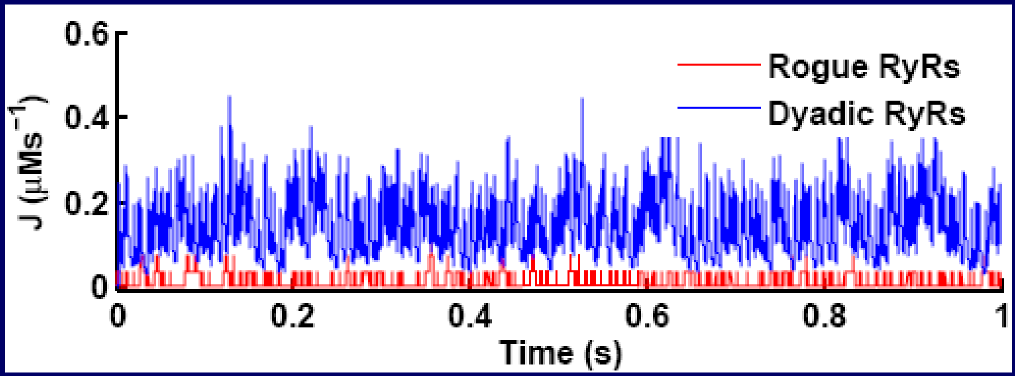


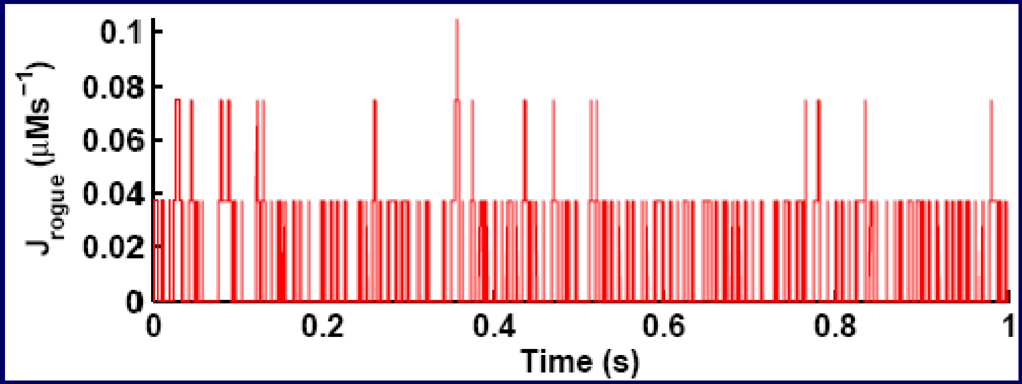


During a calcium transient non-dyadic ryanodine receptors only contribute to a small fraction of the leak

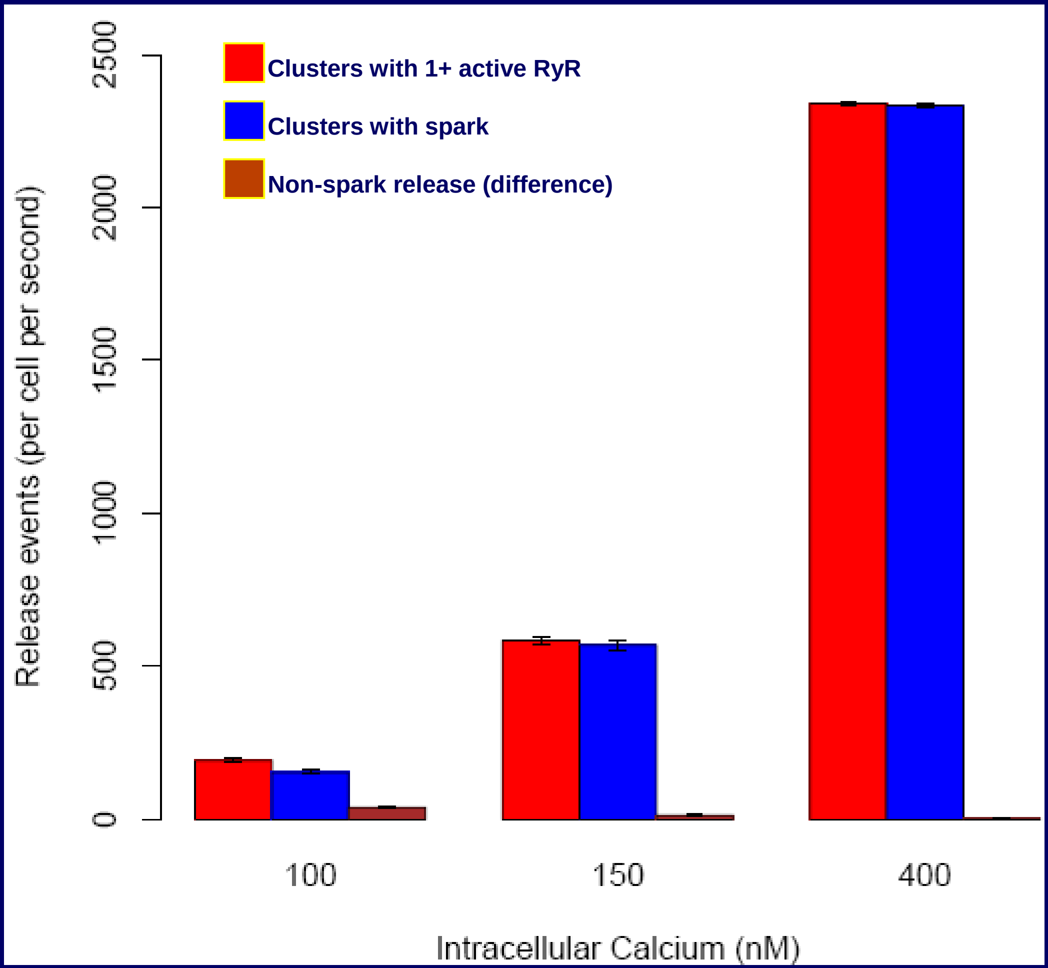
Diastolic Leak

At rest Rogue ryanodine receptors contribute more significantly to leak.



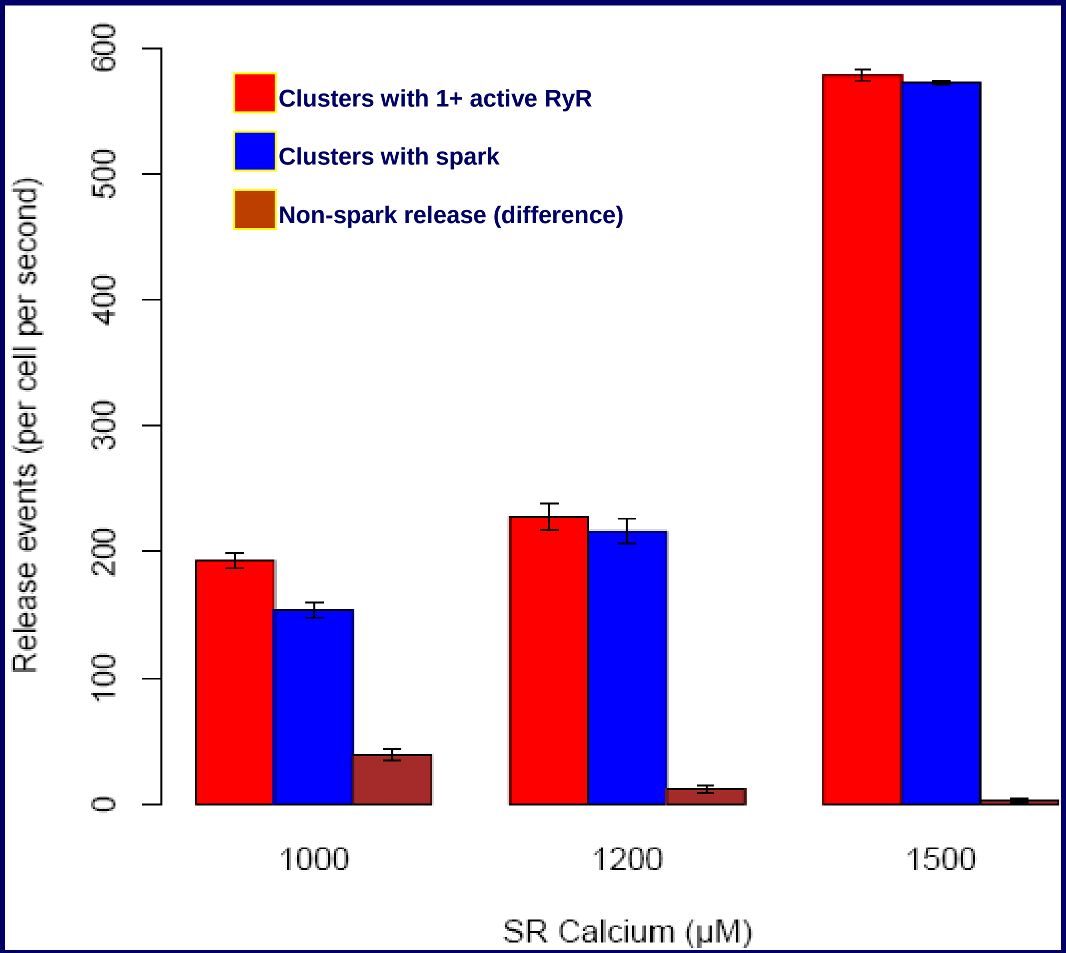


Diadic Leak and Intracellular Calcium



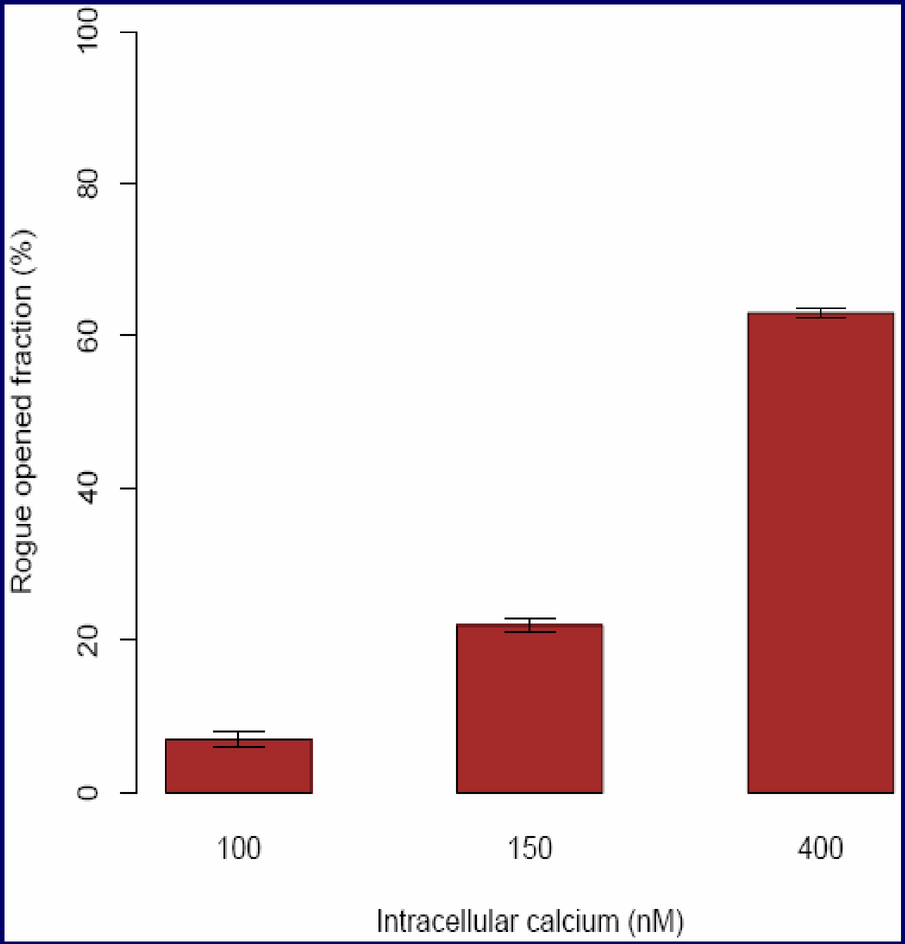
- Total Leak increases with $[Ca^{2+}]_I$
- Non-spark leak disappears
- Cannot measure using Ca overload

Diadic Leak and SR Calcium



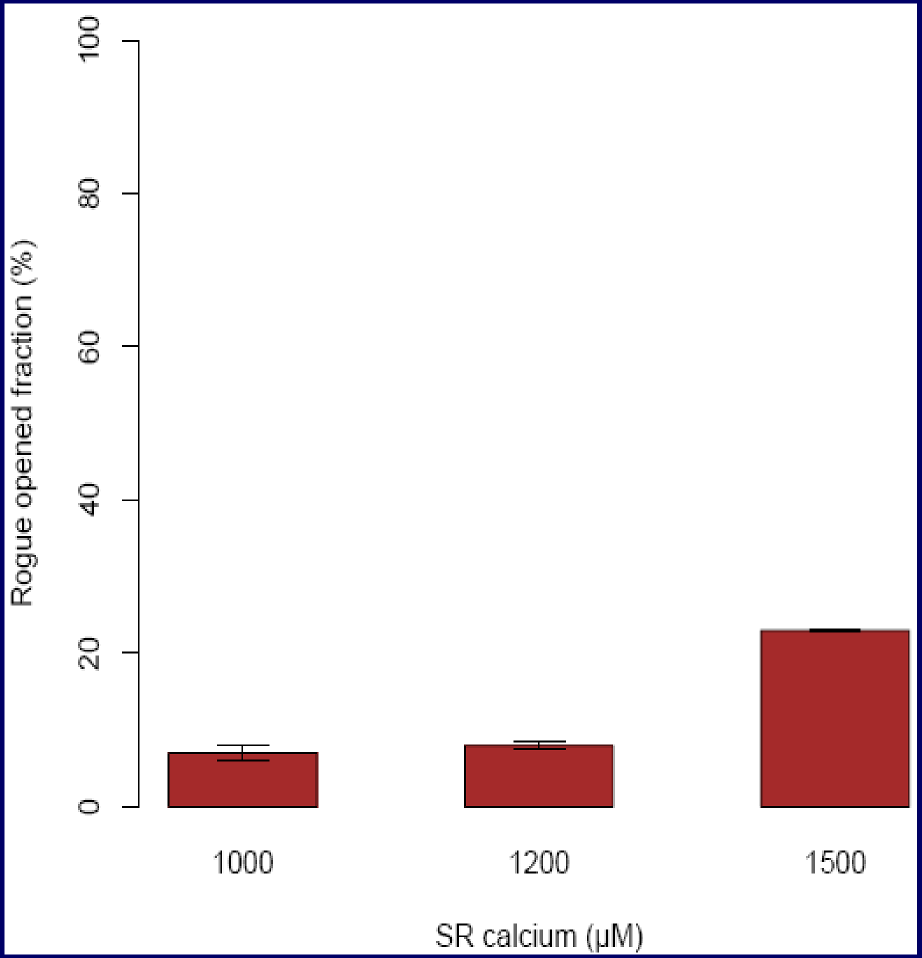
- Total Leak increases with $[Ca^{2+}]_{SR}$
- Non-spark leak disappears
- Cannot measure using Ca overload

Non-dyadic Leak and Intracellular Calcium



- Total Leak increases with $[Ca^{2+}]_i$

Non-dyadic Leak and SR Calcium



- Total Leak increases with $[Ca^{2+}]_{SR}$

Graded Release

- The model simulations supports the hypothesis that summation of Ca^{2+} sparks is sufficient to account for in the global myoplasmic Ca^{2+} transient and provide an SR leak mechanism.
- The model suggests that release in a functional unit is all or none and that graded release occurs through recruitment of different number of functional units.

SR Ca^{2+} Leak

The model suggests that

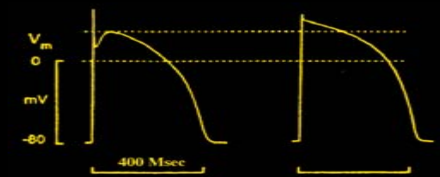
- spontaneous Ca^{2+} sparks can account for the leak of Ca^{2+} out of the SR that serves to balance the SERCA pump at rest.
- there is a non-spark leak at the diad caused by opening of a few RyRs.
- the presence of Rogue RyR can contribute to leak.

Presentation Overview

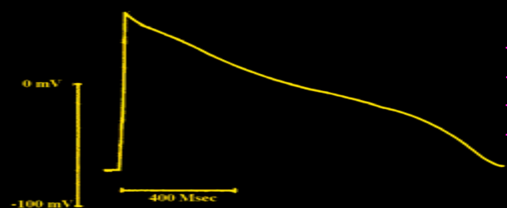
- Introduction
- Common Pool Models
- Calcium Sparks
- Local Control
- Heart Failure
  – Can the current theory explain what is happening?
  – Can we proposed alternate mechanisms?

Cellular Responses in Heart Failure

Epi Endo

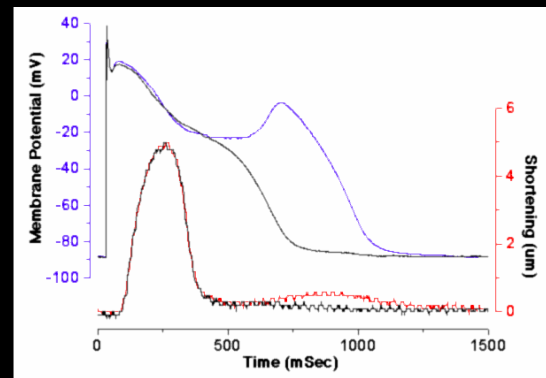


Normal
Human



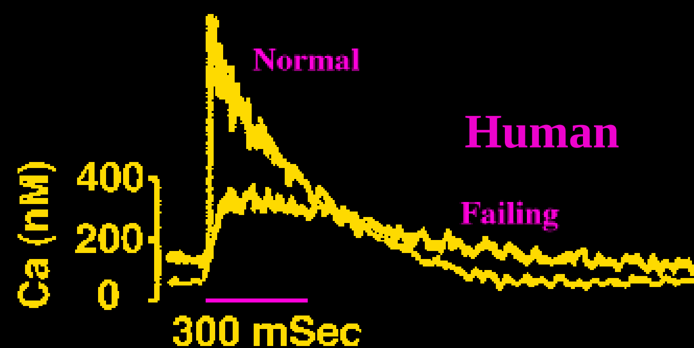
Failing
Human

Beuckelmann et al (1992)
Circulation 85: 1046-1055



Failing
Canine

Nuss et al



Altered K$^+$ Current Expression?

Altered Ca^{2+} Handling?

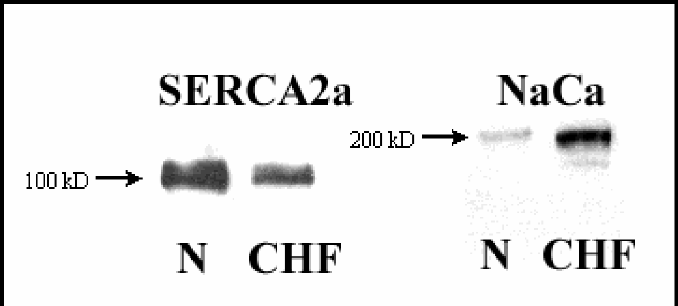
Altered Gene Expression in Canine Tachycardia-Induced Heart Failure

(Kääb et al)

Membrane Currents	I_{to1}	↓	⟷	**66 %**	**Whole-Cell and Patch Recordings**
	I_{K1}	↓	⟷	**32 %**	

(O'Rourke et al)

Ca^{2+} Handling Proteins	$I_{serca2\alpha}$	serca2a	↓	⟷	
	I_{NaCa}	NCX1	↑		



O'Rourke et al

Degree of Up/Down Regulation ?

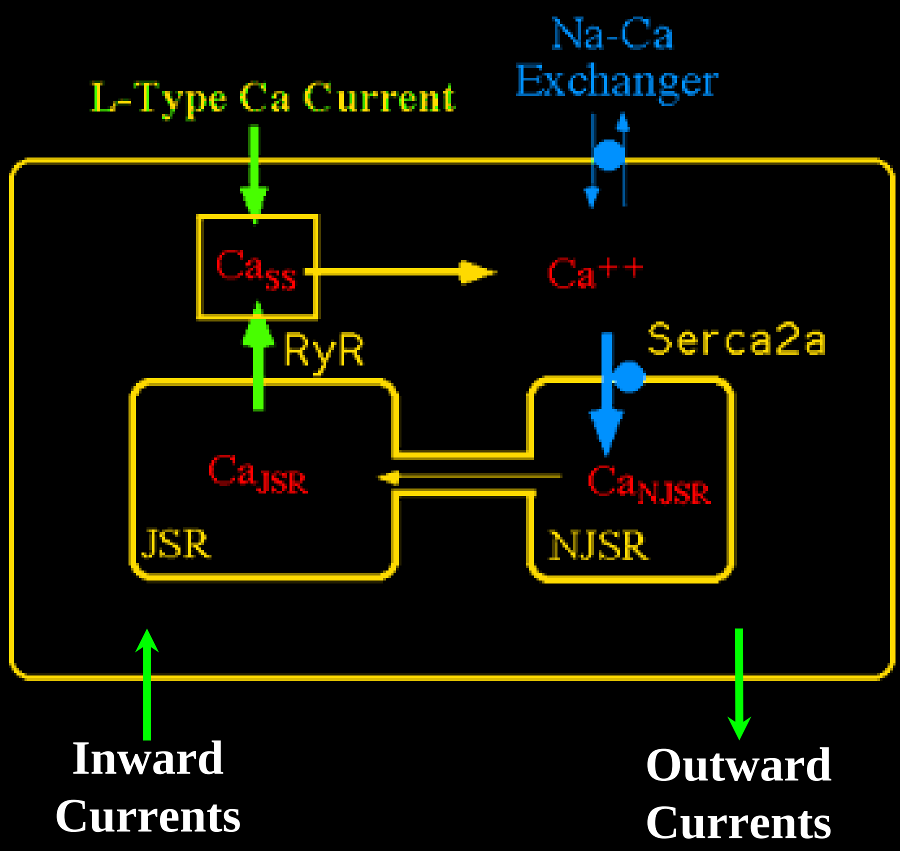
Question

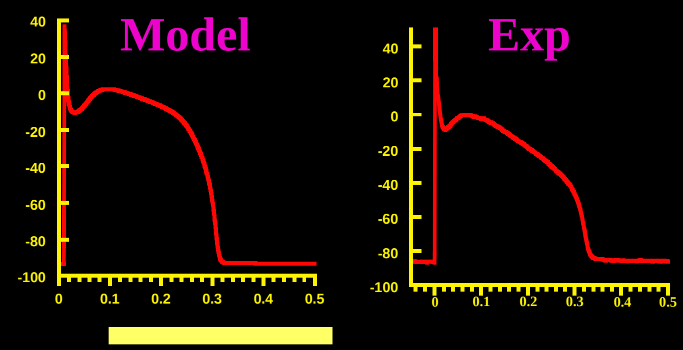
Does the known pattern of altered gene expression account for

- **APD prolongation**
- **altered Ca^{2+} transients**
- **increased risk of arrhythmia**

observed at the cellular level?

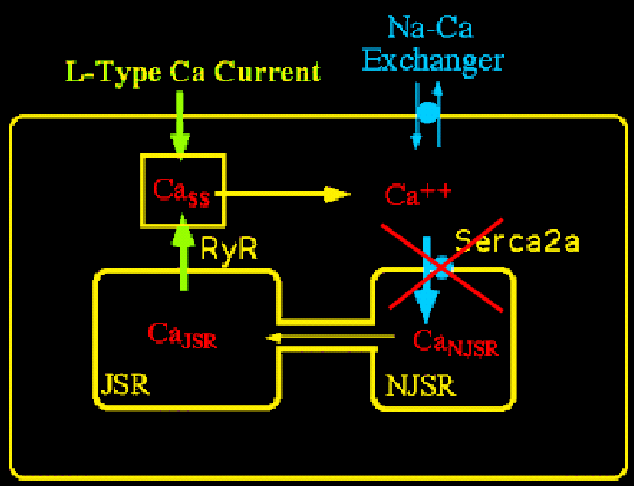
Canine Cardiac Ventricular Cell Model

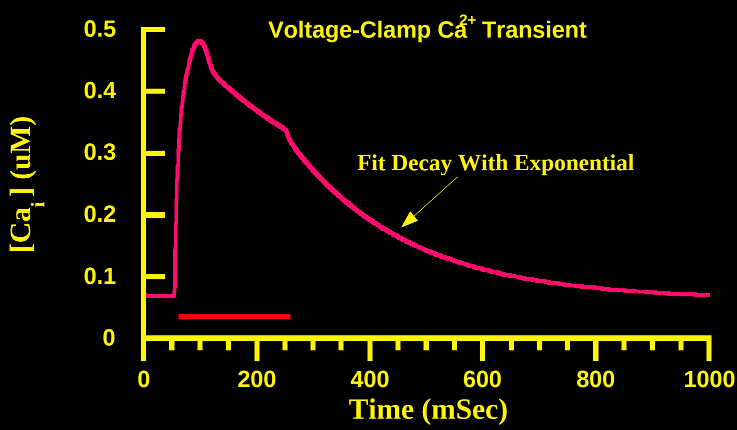


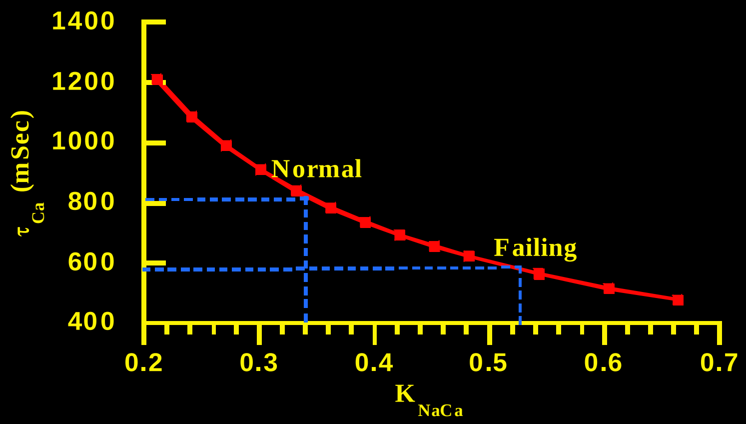


- Origins - LRII Guinea Pig V cell
- Modified I_{Kr}, I_{Ks} I_{K1}
- I_{to1}
- Mode-switching model of Ca-mediated inactivation of $I_{Ca,L}$
- Ca subspace
- Keizer-Levine model of CICR and RyR adaptation

Constraining Na/Ca Exchanger Level: Block serca2a



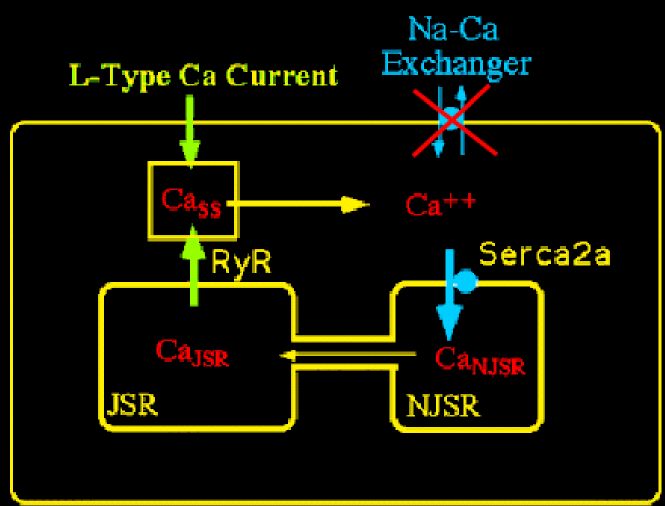


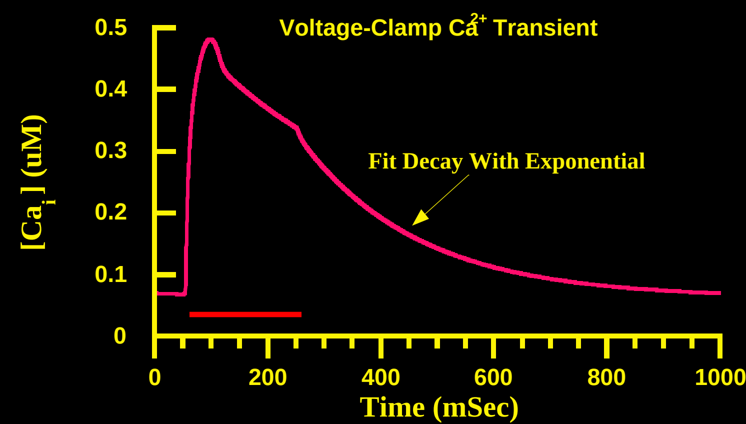


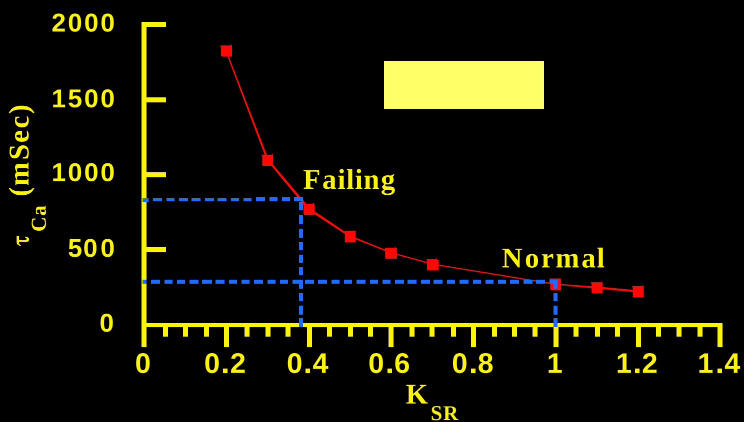
Estimate Na/Ca Exchanger Level In Normal and Failing Cells

75% functional up-regulation

Constraining serca2a Level: Eliminate Na/Ca Exchange





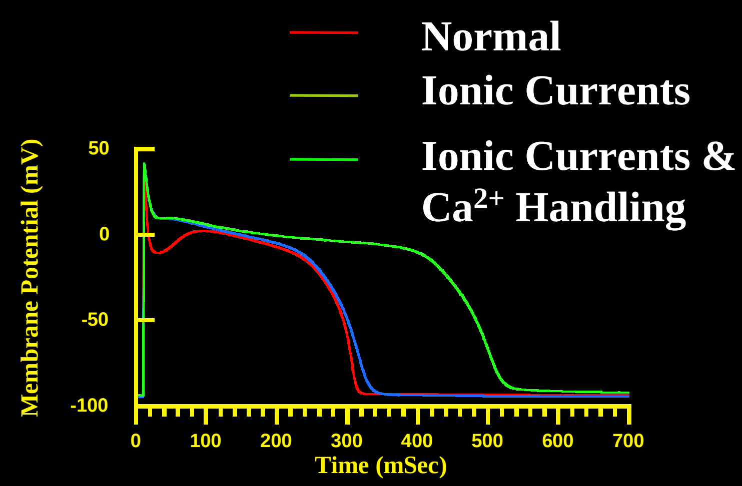


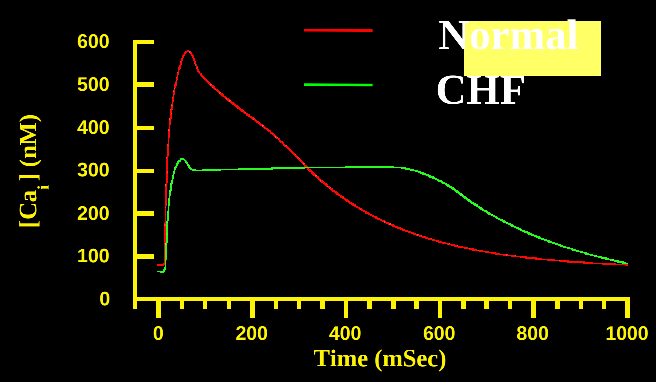
Estimate serca2a Level In Normal and Failing Cells

60% functional down-regulation

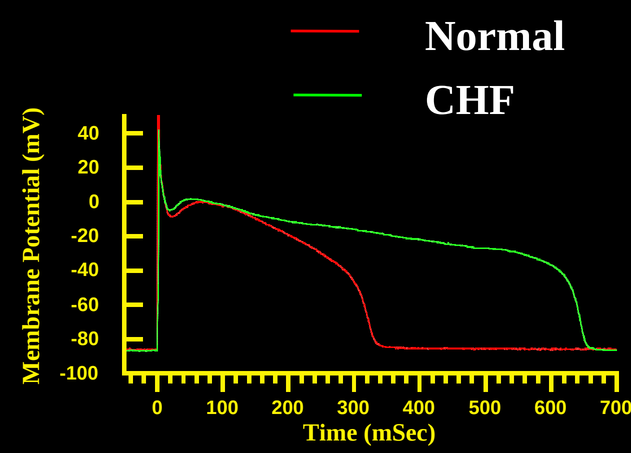
Failing Cell Model

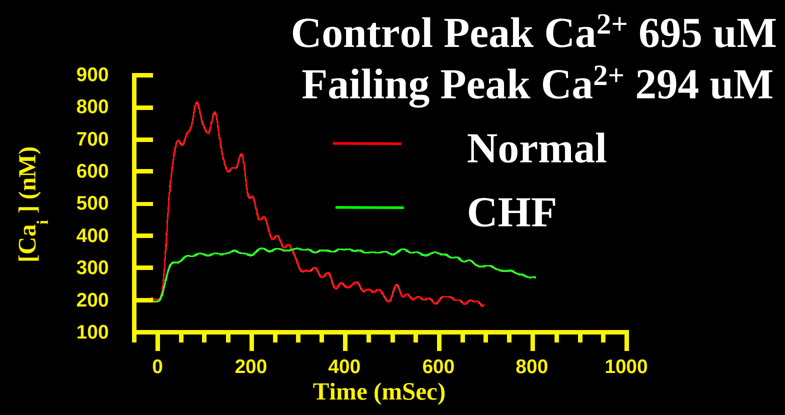
Model





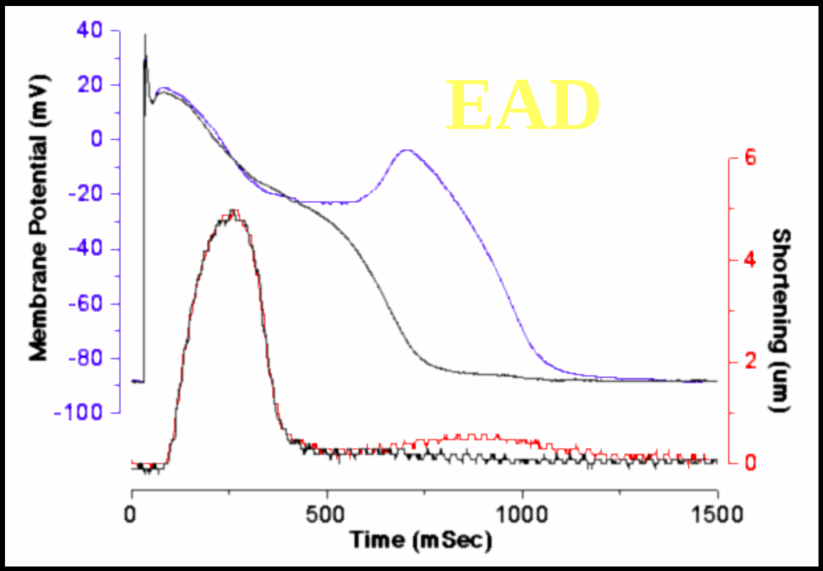
Experiment





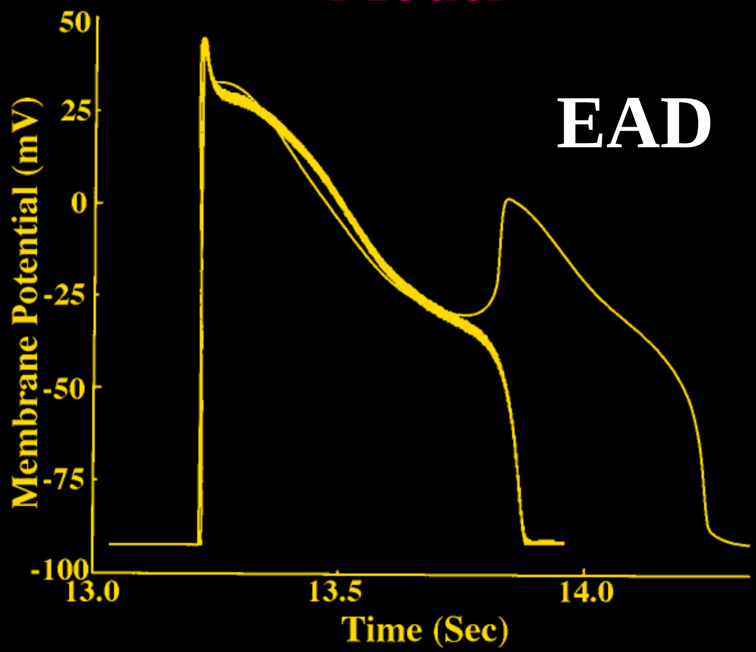
Cellular Arrhythmia

Experiment



Nuss et al

Model



Heart Failure

The model suggests that

- Changes in expression levels of K^+ current alone cannot account for the physiological changes seen in heart failure and
- Changing the calcium dynamics contribute significantly to action potential prolongation and production of EADs.

Acknowledgements

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Greg Smith

Blair Williams

Marco Huertas

IBM

Jeremy Rice

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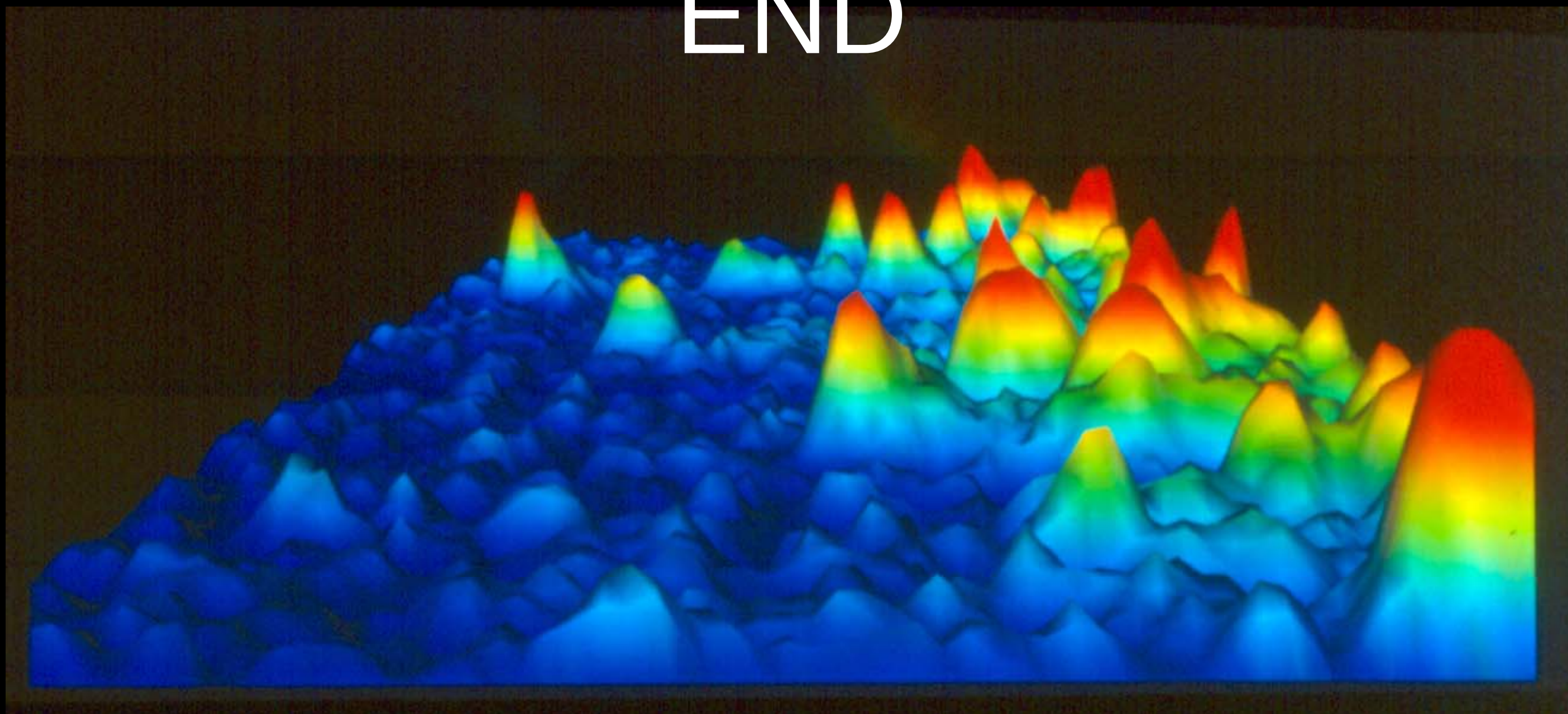
END



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

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