

sarcolemma or nuclear membranes), cytosolic (minimal distance to membranes  $>5\mu\text{m}$ ) or nuclear. Nuclear events were considered membrane associated when occurring at a distance  $<1\mu\text{m}$  from the nuclear envelope or membranes of the nucleoplasmic reticulum. With tetracaine the Ca spark frequency decreased to  $0.7 \pm 0.3$  (from  $8.8 \pm 1.2$  in control), whereas the frequency of events detected after subsequent addition of IP3 increased to  $2.9 \pm 1.3$  (events/ $100\mu\text{m}^2/\text{s}$ ). Subsequent exposure to 2-APB reduced the frequency to  $1.8 \pm 0.8$ . Compared to Ca sparks, local Ca release events in the presence of IP3 were lower in amplitude, prolonged in duration and had a slower rise time. The increase in frequency of local Ca release events was particularly pronounced in the perinuclear regions compared to the cytosol or the subsarcolemmal space. Furthermore, IP3-mediated Ca release events could also be detected within the nucleus. These nuclear events occurred at a higher incidence at locations that were closely associated with membrane structures of the nuclear envelope or the nucleoplasmic reticulum. In conclusion, in atrial myocytes IP3R-mediated Ca release is spatially inhomogeneous and preferentially occurs in the nuclear and perinuclear regions.

#### 2251-Pos Board B270

##### An Integrated Gating Function of Cardiac $\text{Ca}^{2+}$ Release Flux from a Cluster of SR- $\text{Ca}^{2+}$ Channels

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Background: In cardiac cells, sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$ -channels (RyRs) are regrouped into clusters, so-called  $\text{Ca}^{2+}$ -release units (CRUs). Dynamic regulation of the individual RyRs in the cluster determines the characteristics of the SR- $\text{Ca}^{2+}$ -efflux during cell activation. The  $\text{Ca}^{2+}$ -sensitivity of the channel plays a central role in this regulation. However, technical limitations make it difficult to directly assess the mechanism underlying the  $\text{Ca}^{2+}$ -sensitivity of SR- $\text{Ca}^{2+}$ -release in the micro-domain of a CRU. Thus, mathematical models simulating intracellular  $\text{Ca}^{2+}$  activities, such as  $\text{Ca}^{2+}$  sparks and waves, incorporate a  $\text{Ca}^{2+}$ -release function which approximates or, frequently, omits the regulation in the cluster micro-domain. Moreover, using nano-scale components (such as realistic spacing between RyRs in a CRU) in a micro-scale computational domain intending to reproduce the cell geometry requires massive computational resources. Method: To overcome both experimental and computational limitations, we developed a time-dependent  $\text{Ca}^{2+}$ -release function that addresses activation and termination of  $\text{Ca}^{2+}$ -release, mediated by dynamic regulatory mechanisms. This function has been used in systems of ODEs and PDEs applied to cardiac Purkinje cells (Pcells), which included other intracellular modulators of  $\text{Ca}^{2+}$  transients, such as  $\text{Ca}^{2+}$ -buffers and  $\text{Ca}^{2+}$ -pumps. Results: Compared to experimental data from Pcells, the  $\text{Ca}^{2+}$ -release function could predict the instantaneous variation of  $\text{Ca}^{2+}$ -efflux from the CRU. Predicted values for the activation threshold of  $[\text{Ca}^{2+}]$  at the cytosolic side for RyR2 and RyR3 in Pcells were found to be 160 nM-190 nM and 105 nM-110 nM, respectively. These results matched the experimental findings. Conclusion: This function has been used successfully in 1D and 2D numerical models of  $\text{Ca}^{2+}$ -sparks, waves and evoked- $\text{Ca}^{2+}$ -transients in Pcells. This function provides computational support to all modelling addressing the intracellular  $\text{Ca}^{2+}$ -cycling in live muscle cells.

#### 2252-Pos Board B271

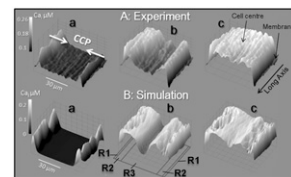
##### Computational Evidence of Centripetal Propagating " $\text{Ca}^{2+}$ -Induced- $\text{Ca}^{2+}$ -Release" in Stimulated Cardiac Purkinje Cells

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**Rationale:**  $\text{Ca}_i$ -variations in Purkinje cells (Pcells) can affect cardiac conduction by generating action potentials in Purkinje fibers thereby promoting arrhythmias. The system underlying  $\text{Ca}_i$ -variations in Pcells is still unknown. Upon stimulation, this system mediates  $\text{Ca}_i$ -elevation propagating from the periphery to the cell centre (Fig.1A).  $\text{Ca}^{2+}$ -diffusion from initial  $\text{Ca}^{2+}$ -release by the peripheral endoplasmic reticulum (ER) could explain this centripetal  $\text{Ca}^{2+}$ -propagation (CCP). Alternatively, study of Pcells suggest a consecutive " $\text{Ca}^{2+}$ -induced- $\text{Ca}^{2+}$ -release" (CICR) from 3 adjacent ER regions expressing distinct  $\text{Ca}^{2+}$ -channels. To determine whether CCP is mediated by diffusion only or "CICR-diffusion-CICR", a system of nonlinear parabolic partial differential equations was developed to simulate  $\text{Ca}^{2+}$ -dynamics in a 2D rectangular array comprising 3 adjacent  $\text{Ca}^{2+}$ -release regions R1, R2, R3 (Fig.1B.b). Simulated CCP was compared against CCP measured in stimulated

porcine Pcells. **Results:** The model accurately reproduced CCP (Fig.1) when CICR was activated in R2 and R3. When CICR was off in R2 and/or R3 and only  $\text{Ca}^{2+}$ -diffusion was considered, CCP collapsed shortly after  $\text{Ca}^{2+}$ -release in R1. **Conclusion:** our results are consistent with a mechanism of consecutive ER- $\text{Ca}^{2+}$ -releases propagating centripetally by CICR-diffusion-CICR. The intermediate region R2 appears to be a critical functional link in the process.



#### 2253-Pos Board B272

##### Synchronization of $\text{Ca}^{2+}$ Release in Multicellular Cardiac Preparations

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Delayed afterdepolarizations (DADs) are frequently observed under conditions that increase cellular and SR  $\text{Ca}^{2+}$ . The high  $\text{Ca}^{2+}$  can cause spontaneous SR  $\text{Ca}^{2+}$  release and oscillations in the membrane potential and evoke spontaneous action potentials in the myocytes, leading to arrhythmias. Since myocytes are electrically coupled, any depolarization that arises in one myocyte would be dampened due to dissipation of the current in the neighboring myocytes. It is believed that some synchronization mechanism exists that synchronizes DADs and action potentials in neighboring cells. In our current study, we are interested in examining whether there is a synchronization mechanism for the generation of triggered activity in multicellular cardiac preparations. Trabeculae isolated from rat hearts were mounted on a modified confocal microscope that was equipped with a force transducer. Trabeculae were loaded with Rhod-2 AM and  $\text{Ca}^{2+}$  imaging was performed. Combination of caffeine and isoproterenol were added to the perfusion to induce extra-systolic contractions (ESCs). Line scans were acquired and fluorescence emitted was expressed as  $\Delta F/F_0$ . The addition of caffeine and isoproterenol show an increased incidence of ESCs in our force experiments. The amplitude of these ESCs ranged anywhere from 10% to 100% of the previous, stimulated contraction's amplitude. We hypothesize that many of these ESCs would likely result in an afterdepolarization. In the  $\text{Ca}^{2+}$  experiments, we noticed that with the addition of both caffeine and isoproterenol, we could visualize  $\text{Ca}^{2+}$  waves in the multicellular preparation. While these  $\text{Ca}^{2+}$  waves were not always present in every myocyte within the trabeculae initially, we were able to see that  $\text{Ca}^{2+}$  waves can, indeed, synchronize in multiple cells within the muscles over time. Therefore, these results suggest that intracellular, diastolic SR  $\text{Ca}^{2+}$  release can become synchronized in a multicellular cardiac preparation.

## Lipid Signaling

#### 2254-Pos Board B273

##### Improved Models of Short-Chain Phosphoinositide Efficacy on Ion Channels

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In determining the efficacy of phosphoinositides on ion channels in inside-out patches, we and others typically measure channel activity as a function of the solution concentration of short-chain phosphoinositides such as di-C8-PI(4,5)P<sub>2</sub>. However, channels are believed to bind phosphoinositides *not* from solution, but from the phosphoinositide lipids incorporated into the plasma membrane. The extent to which different phosphoinositide lipids partition into membranes is expected to vary widely depending on charge, chain length, and head-group structure, such that the same solution concentration of different phosphoinositides will produce very different concentrations in the plasma membrane. We have previously shown that the polymodal ion channel TRPV1 has a higher apparent affinity for di-C8-PI(4,5)P<sub>2</sub> than for di-C8-PI(4)P. We re-examined this apparent PI(4,5)P<sub>2</sub> selectivity using a model that incorporates an established description of phosphoinositides' partition between solution and the lipid membrane. We found that the data could not distinguish between two very different interpretations: that the channel itself is activated more easily by di-C8-PI(4,5)P<sub>2</sub> than di-C8-PI(4)P; or that di-C8-PI(4,5)P<sub>2</sub> and di-C8-PI(4)P are equally effective in activating TRPV1, but for a given phosphoinositide lipid solution concentration, there is more di-C8-PI(4,5)P<sub>2</sub> in the membrane than there would be di-C8-PI(4)P. We further tested our model on a channel mutant that appears to invert the selectivity for di-C8-PI(4,5)P<sub>2</sub> and di-C8-PI(4)P. Our examples have wide ranging implications for the interpretation of dose-response relations of any ligand that binds to lipid membranes, especially when comparing the efficacy of different membrane binding ligands.