incubation of saponin-permeabilized VM (confocal imaging of Fluo-4 saltfluorescence) with a PP inhibitor (CyA 0.5 μM) or/and PDE inhibitor (IBMX 10μM) converts stochastic sparks into periodic, spatially synchronized local Ca^{2+} releases (Table). Moreover, the combination PP and PDE inhibitors produced rhythmic, local Ca^{2+} releases with a shorter period than that produced by either of inhibitors alone.

Thus, VM have an inherent capacity to self-organize spontaneous basal RyR activation and synchronize Ca^{2+} release, but this is physiologically suppressed by constitutive PDE and PP activity.

Local Ca²⁺ Release Characteristics	Control, [Ca²⁺], 100nM (n=7)	+ 0.5μM CyA (n=4)	+ 10μm IBMX (n=3)	+ 0.5μM CyA+10μm IBMX (n=7)
Amplitude (ΔCa²⁺, nM)	41.4± 2.5	44.9± 2.4	51.9±3.2	56.3± 3.6*
Size (μm)	3.1±0.1	3.6±0.1	4.0±0.3*	4.2±0.2*
Duration (ms)	29±1.5	40.6±3.8	39.7± 3.6*	45.6±2.3*
Number (N, 1s×100μm of line scan)	29.2±4.7	54.4±6.3	68.7±9.8*	83.9±3.6*
Average signal mass (μm×ms×1/ΔCa²⁺)	2125.2±278.6	3924.8±289.9*	4534.4±1098.7*	6198±645.8*
Total signal mass (N×μm×ms×1/ΔCa²⁺)	26188.0±4199.6	81222.5±10274.9*	116335.5±10333.1*	206690.7±22009.4*
Rhythmicity (frequency, Hz)	·	3.0±1.0	2.4±0.6	3.2±0.4
% of cells with periodicity of Ca²⁺ release	0.0	50.0	67.0	71.0

Repeated Measures ANOVA, *P<0.05 vs Control
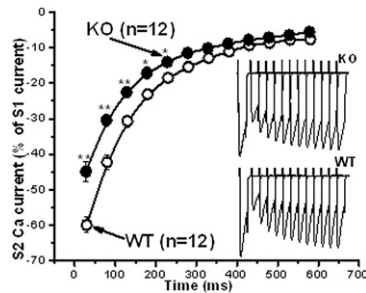
3412-Pos Board B273
Accelerated Recovery of L-Type Ca Current Contributes to the Loss of SR Ca Release Restitution in Mice Lacking Casq2
Dmytro Kryshtal, Oleksiy Gryshchenko, Björn C. Knollmann.
Vanderbilt University, Nashville, TN, USA.
Cardiac Ca-release from sarcoplasmic reticulum (SR) is reduced with successively shorter coupling intervals of premature stimuli, a phenomenon known as SR Ca-release restitution. Interestingly, myocytes lacking the SR luminal Ca binding protein calsequestrin (Casq2 KO) lack SR Ca-release restitution. Here we test whether altered L-type Ca current (I_{Ca}) responsible for triggering SR Ca-release contributes to the loss of Ca-release restitution.
Results: I_{Ca} was recorded in voltage-clamped ventricular myocytes isolated from Casq2 KO mice and wild-type (WT) littermates. Cells were pre-treated with ryanodine and thapsigargin to eliminate SR Ca-release. After a 1 Hz train of depolarizing stimuli (S1), I_{Ca} restitution was measured by applying premature stimuli (S2) at successively shorter S1-S2 coupling intervals. Both groups have decreased I_{Ca} at short S1-S2 intervals, but I_{Ca} recovered significantly faster in KO vs. WT myocytes (p<0.001, **Figure**). Eliciting Ca-release by I_{Ca} tail currents (which eliminates participation of I_{Ca} restitution) reduced the difference between WT and KO Ca-release restitutions.
Conclusion: Accelerated recovery of I_{Ca} contributes to loss of SR Ca-release restitution in mice lacking Casq2 and may further increase risk for ventricular arrhythmia in vivo.

3413-Pos Board B274
Calcium Mediated Triggered Activity in Cardiac Tissue
Yohannes Shiferaw, Wei Chen, Mesfin Asfaw.
California State University, Northridge, Northridge, CA, USA.
It is well known that various cardiac arrhythmias are initiated by an ill-timed excitation that originates from a focal region of the heart. However, up to now, it is not known what governs the timing, location, and morphology of these triggered beats. Recent studies have shown that these focal excitations can be caused by abnormalities in the calcium (Ca) cycling system. However, the cause and effect relationships linking subcellular Ca dynamics and triggered activity in cardiac tissue is not completely understood. In this paper we present a minimal model of Ca mediated triggered activity in cardiac tissue. This model accounts for the stochastic nature of spontaneous Ca release on a one dimensional cable of cardiac cells. Using this model we show that the timing of triggered activity is equivalent to a first passage time problem in a spatially extended system. In particular, we find that for a short cable the mean first passage time (MFPT) increases exponentially with the number of cells in tissue, and is critically dependent on the ratio of inward to outward currents near the threshold for an action potential (AP). For long cables triggered activity occurs due to ectopic foci which occur on a length scale determined by the minimum length of tissue that can induce an AP. Furthermore, we find that for long cables the MFPT decreases as a power law in the number cells. These results provide precise criteria for the occurrence of focal excitations in cardiac tissue, and will serve as a guide to determine the propensity of Ca mediated triggered arrhythmias in the heart.

3414-Pos Board B275
A Validated Computational Model of Cardiac Pacemaking: Mechanisms of Physiological and Pharmacological Rate Modulation
Stefano Severi[1], Matteo Fantini[1], Enrico Ravagli[1], Lara Charawi[2], Dario DiFrancesco[3].
[1]Department of Electronics, Computer Science and Systems, University of Bologna, Cesena, Italy, [2]Department of Mathematics, University of Milano, Milano, Italy, [3]Department of Biomolecular Sciences and Biotechnology, University of Milano, Milano, Italy.
The cellular basis of heart's pacemaking activity, and specifically the degree of contribution of the different mechanisms involved, is still debated. Reliable computational models of the sinoatrial (SAN) action potential (AP) may help gain a deeper understanding of the phenomenon. Recently, novel models incorporating a detailed calcium-handling dynamics have been proposed, but they fail in reproducing experimental effects of "funny" current (If) reduction. We therefore developed a SAN AP model, based on available experimental data, to reproduce and investigate autonomic and drug-induced rate modulation.
Cell compartmentalization and all the intracellular $Ca2+$ handling mechanisms were formulated as in the Maltsev-Lakatta model. Membrane current equations were revised on the basis of published experimental data. Modifications to the current formulations to simulate autonomic modulation and drug effects (Acetylcholine, Isoprenaline, Ivabradine, Cesium, BAPTA, Ryanodine) were derived from experimental data.
The model generates AP waveforms typical of rabbit SAN cells, whose parameters fall within the experimental ranges: 176ms AP duration, 329ms cycle length, 73mV AP amplitude, -56mV maximum diastolic potential and 6.23V/s maximum upstroke velocity. Rate modulation by If-blocking drugs agrees with experimental findings: 18% and 20% Cesium-induced (5mM) and Ivabradine-induced (3μM) rate reductions, respectively. The model reproduces the autonomic modulation effects, with Acetylcholine- and Isoprenaline-induced rate variations, in a way that is quantitatively consistent with experimental data. Model testing of Ryanodine and BAPTA effects showed slowing of rate without cessation of beating.
Our up-to-date model describes satisfactorily experimental data concerning autonomic stimulation, funny-channel blockade and inhibition of the Ca-related system by specific drugs, making it a useful tool for further investigations. Simulation results suggest that a detailed description of the intracellular calcium fluxes is fully compatible with the observation that If is a major component of pacemaking and rate modulation.

3415-Pos Board B276
Contribution of Store-Operated Ca^{2+} Entry to Beat-to-Beat Ca^{2+} Transient in Early Fetal Mouse Heart
Takayuki Kurokawa[1], Takahiro Ishiwata[1], Takashi Kanai[1], Hideyuki Ishida[2], Kaori Sekine[2], Naoko Ishibashi[1], Yu Asano[1], Shigeaki Nonoyama[1].
[1]Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan, [2]Department of Physiology, Tokai University, School of Medicine, Isehara, Japan.
Introduction: Store-operated Ca^{2+} entry (SOCE) is triggered by ER/SR Ca^{2+} depletion to maintain intracellular Ca^{2+} concentration. In adult myocytes, SOCE contributes to cell growth or hypertrophy. However, in fetus, little is known about the role of SOCE. We hypothesize that SOCE may contribute to fetal cardiac contraction. **Method:** The whole hearts in embryonic day(ED) 9.5/12.5/15.5 and neonate were stained by Fluo-3 AM. Fluorescence signals in beating hearts stimulated electrically were recorded using a photomultiplier, then analyzed as Ca^{2+} transient(CaT) in each age. 1) SOCE was induced by changing the solutions from zero to 1.8mM Ca^{2+}. With inhibiting voltage-dependent Ca^{2+} channels(VDCC), we tested whether SOCE was suppressed by 0.2mM Gd^{3+} application, as SOCE blocker. 2) We tested whether beat-to-beat CaT was suppressed by 0.2mM Gd^{3+} application. **Results:** 1) In fetal heart, SOCE was suppressed significantly by Gd^{3+} application (ED9.5; 46% decreased, p=0.011, n=5. ED12.5; 82% decreased, p<0.0001, n=6). 2) In ED 9.5, CaT was depressed significantly by Gd^{3+} application (49.2+/-7.6% vs. 2.0+/-2.0%), but after ED 12.5, CaT was not depressed. **Conclusion:** SOCE would contribute to beat-to-beat CaT in early fetal mouse myocytes.