diastolic depolarization and may contribute to the periodic excitations of pacemaker cells [Vinogradova et al., Circ. Res. 02]. Such intracellular Ca^{2+} oscillations, referred to as Ca^{2+}-"clock" [Maltsev, Lakatta, Am J Physiol Heart Circ Physiol, 2009], interact with the classic sarcolemmal voltage oscillator (membrane clock) by activating Na^{2+}-Ca^{2+} exchange current.
We developed an Electron-Conformational Model (ECM) of the stochastic RyR-channel activity, which accounted for cooperative effects of interaction between coupled RyR-channels within a Ca^{2+} release unit (RU) in cardiac pacemaker cells [Moskvin et al., PBMB 2006]. The RU includes a junctional Ca^{2+} release compartment of the SR network, a cluster of coupled RyR-channels, and adjacent sarcolemmal subspace. Inter-channel conformational coupling between nearest RyR neighbors is accounted for the energy potential of the cluster. The RU activity is described along with intracellular Ca^{2+} cycling between the subspace, cytosol, and the SR network.
Computer simulations of a 9x9 RyR square lattice within the framework of ECM demonstrate that the Ca^{2+} SR "clock" activity is regulated by the interaction between RyR channels. Stability of oscillatory dynamics, frequency and amplitude of Ca^{2+} auto-oscillations depend essentially on the RyRs coupling force. We found that a combination of rather low Ca^{2+} release rate with strong enough RyR-RyR coupling may trigger sudden break of Ca^{2+} oscillations by forming of stable clusters of opened RyRs and thus providing with the steady-state Ca^{2+} leakage from the SR.
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Formation of Subcellular Calcium Waves in Cardiac Myocytes: Characterizing Timescales via Mathematical Modeling
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Subcellular Calcium (Ca) cycling plays fundamental roles in normal heart dynamics. In cardiac myocytes, the elementary Ca cycling events are Ca sparks: random discretized Ca release events due to random and collective openings of the ryanodine receptor (RyR) channels clustered in Ca release units (CRUs). A typical cardiac myocyte includes about 10,000 to 20,000 CRUs, and the spatial arrangement of CRUs varies widely across myocyte type and changes in diseased conditions. Dysfunction of the CRU network leaves cells prone to subcellular Ca waves, notorious triggers of highly arrhythmogenic delayed afterdepolarizations. Recent experimental studies have isolated three timescales involved in the formation of Ca waves: rate of sarcoplasmic reticulum (SR) Ca reuptake, intrinsic RyR refractoriness, and a so-called "idle" period. Here we use a physiologically detailed computational model of a spatial and stochastic CRU network to study the variables that contribute to the aforementioned timescales, and identify how the relative dominance of each affects the morphology of Ca waves. We show that the "idle" period is far from idle, as it emerges out of complex Ca mediated CRU-to-CRU interactions in both the myoplasm and SR. We also find that while reduced refractory period and increased SR Ca diffusion enhance the local initiation of waves, they hinder propagation, resulting in fractionated wave events. Furthermore, at very short refractory periods the system degenerates into spiral waves and chaos. Pinpointing the mechanisms underlying the variety of observed wave morphologies is an important step in our understanding of diseased states, as each may play a different role in arrhythmogenesis.

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Spatial Imperfection Encodes Functional Perfection: Success and Failure of Calcium Release to Propagate Regulate Pacemaker Cell Function
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Local Diastolic Ca^{2+}releases (LCRs) contribute to sinoatrial-node-cell pacemaker function by activation of Na^{+}/Ca^{2+}exchanger current that accelerates diastolic depolarization. LCRs are generated by clusters of ryanodine receptors, Ca^{2+}release units (CRUs), residing in the sarcoplasmic reticulum. CRU distribution in pacemaker cells exhibits substantial heterogeneity vs. a crystal-like distribution of cardiac muscle cells. While this heterogeneity can be noticed in images of ryanodine receptor immunofluorescence, its functional importance has never been addressed. We approached the problem by performing numerical simulations in a new sinoatrial-node-cell model that has membrane ion currents and a sub-membrane sarcoplasmic reticulum with a Ca^{2+}pump and two-dimensional array of stochastic, diffusively coupled CRUs. With an equally-spaced, crystal-like distribution of CRUs, synchronization of Ca^{2+}releases during diastolic depolarization undergoes an explosive, phase-like transition from individual Ca^{2+}sparks to simultaneous occurrence of merging numerous LCRs over the entire cell, i.e. an all-or-none behavior. Since almost all CRUs fire during the phase transition (leaving no reserve), the model is not amenable to autonomic modulation of pacemaker rate. Allowing each CRU position to vary around its original crystalline position fundamentally changes the model behavior: the transition from individual sparks to propagating LCRs becomes smooth with the number and sizes of LCRs observed experimentally. Furthermore, some distant CRUs do not fire during diastolic depolarization under resting conditions, but become engaged (as a reserve) during beta-adrenergic stimulation. Thus, the model with the CRU positional variability is easily tuned to respond to autonomic modulation. We conclude: the irregularity in CRU distribution in sinoatrial-node-cells is not a noise or imperfection, but rather a functional modality of the pacemaker cells that allows them to gradually engage CRUs to satisfy the chronotropic demand of the heart at a given condition.

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Calcium Signaling in Muscle Cells from a Patient Cohort with Inheritable Muscle Diseases
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Malignant Hyperthermia Susceptibility, MHS, is a hereditary sub-clinical condition characterized by events of uncontrolled release of calcium, deadly if untreated. 60% of MHS cases are due to mutations of the RyR1 Ca release channel. A puzzling property of these mutations is their phenotypic variability. We are comparing Ca signals in cells from MHS patients with those from normal donors. Every subject is genotyped for the proteins of interest. *Gracilis* muscle biopsies taken in Toronto from at risk patients, are received in Chicago overnight and studied acutely with 2-silicone gap voltage clamp, or used to generate cultures (technique by S Treves, Basel). Myotubes are imaged for $[Ca^{2+}]_{cyto}$ (with fluo-4) and V_m (with di-8-ANEPPS). In response to brief field stimuli, cells from healthy subjects had normal action potentials (t ~10 ms) and a monophasic Ca transient, rarely followed by a slow wave. Cells from a patient with the MH-linked mutation G341R always had a 2-phase response –a small initial transient followed by a large propagated wave. Cells from two MHS patients with normal genotype (who were positive for one of 2 standard MH tests) had responses with intermediate features. Using pharmacology and photorelease of Ca^{2+} and IP3, we are testing various mechanisms that could be altered, including gating control of RyRs as well as IP3 receptor channels, which contribute to slow transients in myotubes (e.g. Casas et al., 2010). Given the variety of phenotypes and genotypes available, these approaches should allow the characterization of cellular signaling defects in multiple mutations, linked to MH and other muscle diseases. The comparison should increase our understanding of disease mechanisms as well as our insight on structure-function relationships of the RyR channel. Funded by NIAMS/NIH and A.H.A.

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Ca^{2+} Nanosparks: Junctional Ca^{2+} Dynamics Probed with a New Targeted Biosensor
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In cardiac dyads, junctional Ca^{2+} directly controls the gating of the ryanodine receptors (RyRs), and is itself dominated by RyR-mediated Ca^{2+} release from the sarcoplasmic reticulum. Being able to directly probe junctional Ca^{2+} dynamics should increase our understanding of cardiac excitation-contraction coupling and its modification in disease states. We developed a new targeted calcium biosensor based on the latest generation of fluorescent protein probes, GCaMP6f by fusing it to triadin and/or junctin. This probe targeted the dyad as shown by colocalization with RyRs after adenovirus-mediated transfection in single cardiac myocytes from adult rat. This biosensor had faster kinetics and slightly elevated K_d compared to native GCaMP6f. Confocal imaging revealed high-contrast biosensor fluorescent transients ("Ca^{2+} nanosparks") that occupied a ~50-times smaller volume than conventional Ca^{2+} spark records obtained with diffusible indicators. These spatially discrete signals showed no diffusive component outside the labeled region, allowing high-contrast recordings of dyad activation. The maintenance of high dyad-to-background contrast amidst whole cell Ca^{2+} transients allowed us to detect the latency and duration of individual dyad activation during normal