The paradigm shift: heartbeat initiation without the pacemaker cell

Victor A. Maltsev, Michael D. Stern*

Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, NIH, 251 Bayview Blvd, Baltimore, Maryland 21224

***Corresponding author:**

Michael D. Stern

Email: SternMi@mail.nih.gov

Abstract

The current dogma about the heartbeat origin is based on the pacemaker cell, a specialized cell residing in the sinoatrial node (SAN) that exhibits spontaneous diastolic depolarization triggering rhythmic action potentials (APs). Recent high-resolution imaging, however, demonstrated that Ca signals and APs in the SAN are heterogeneous, with many cells generating APs of different rates and rhythms or even remaining non-firing (dormant cells), i.e. generating only subthreshold signals. Here we numerically tested a hypothesis that a community of dormant cells can generate normal automaticity, i.e. the pacemaker cell is not required to initiate rhythmic cardiac impulses. Our model includes (i) non-excitable cells generating oscillatory local Ca releases and (ii) an excitable cell lacking automaticity. While each cell in isolation was not the pacemaker cell, the cell system generated rhythmic APs: the subthreshold signals of non-excitable cells were transformed into respective membrane potential oscillations via electrogenic Na/Ca exchange and further transferred and integrated (computed) by the excitable cells to reach its AP threshold, generating rhythmic pacemaking. Conclusions: Cardiac impulse is an emergent property of the SAN cellular network and can be initiated by cells lacking intrinsic automaticity. Cell heterogeneity, weak coupling, subthreshold signaling, and their summation are critical properties of the new pacemaker mechanism, i.e cardiac pacemaker can operate via a signaling process basically similar to that of temporal summation happening in a neuron with input from multiple presynaptic cells. The new mechanism, however, does not refute the classic pacemaker cell-based mechanism: both mechanisms can co-exist and interact within SAN tissue.

Keywords: Heartbeat, pacemaker mechanism, diastolic depolarization, local calcium release, sinoatrial node.

Introduction

During a normal life span the human heart executes more than 2 billion rhythmic contraction cycles, pumping blood to all organs commensurate with body demand at any given condition. Our understanding of the heartbeat origin has been evolving with the advances in technology, experimental techniques, ideas, and computation power. A major milestone in 1907 was the discovery of the sinoatrial node (SAN), a small piece of specialized heart tissue that paces heart contractions (Keith and Flack, 1907). In 1937, a slow potential change (i.e. the diastolic depolarization in current terminology) preceding the discharge of cardiac impulses was discovered by Arvanitaki working on the snail heart (Arvanitaki et al., 1937). Based on Hodgkin-Huxley formalism, Noble (Noble, 1962) explained the origin of the diastolic depolarization of "the pacemaker cell" as an interplay of kinetics of membrane ion currents. Further studies proposed a critical contribution of intracellular Ca cycling in addition to the membrane ion channels, giving rise a modern theory of the coupled-clock system (Maltsev and Lakatta, 2009) or ignition process via positive feedback mechanisms among local Ca releases (LCRs), Na/Ca exchanger (NCX) and L-type Ca channels (particularly of Cav1.3 isoform) (Lyashkov et al., 2018;Maltsev et al., 2022b).

At the SAN tissue level, it was initially thought that the pacemaker cell or a leading pacemaker center dictates the excitation rate and rhythm of other SAN cells (Sano et al., 1978;Bleeker et al., 1980). Subsequent studies suggested, however, that individual SAN cells mutually entrain each other to fire action potentials (APs) with a common period but different phases (dubbed "democratic" process) resulting in "apparent" propagation (Jalife, 1984;Michaels et al., 1987). Electrical mapping and imaging of SAN tissue demonstrated concentric excitation propagation from a leading center to SAN periphery (Efimov et al., 2004;Lang et al., 2011;Li et

al., 2017). Thus, regardless of the propagation is real or apparent, the present dogma is that the pacemaker cell (possessing intrinsic automaticity) or a group of such cells with the fastest spontaneous diastolic depolarization rate within the SAN tissue paces or entrain other SAN cells and the entire heart.

While the spontaneously beating pacemaker cell(s) is thought to initiate cardiac impulse, only 10-30% of isolated cells actually contract spontaneously (Nakayama et al., 1984). The remaining nonfiring cells could not be ascribed as the true pacemaker cells and were not studied. Also, there has been always an uncertainty whether these cells were damaged by the enzymatic isolation procedure. Recent studies, however, demonstrated that this major population of non-firing cells (dubbed dormant cells) is not "dead", but generates subthreshold oscillatory signals and many dormant cells can be reversibly "awakened" to generate normal automaticity in the presence of β-adrenergic receptor stimulation (Kim et al., 2018;Tsutsui et al., 2018;Tsutsui et al., 2021;Louradour et al., 2022). Dormant cells isolated from different locations (superior vs. inferior regions of SAN) demonstrated different properties of noise of Ca signals and membrane potential (V_m) subthreshold oscillations (Grainger et al., 2021), indicating that the role of these cells differ in different SAN locations.

While earlier low-resolution imaging of SAN tissue showed concentric continuous spread of excitation propagation, recent studies performed at a higher (individual cell) resolution level discovered a major fraction of non-firing cells (Bychkov et al., 2020;Fenske et al., 2020), and the tissue excitation within the SAN center appeared to be discontinuous, consisting of functional cell communities (presumably functional modules) with different firing patterns (Bychkov et al., 2020). Ca signals and APs in the communities are highly heterogeneous, with many cells generating APs of different rates and rhythms or remaining non-firing, generating subthreshold

signals (Video 1). Thus, a possible functional role of non-firing cells and their subthreshold signals represents an open problem in cardiac biology.

One idea is that synchronized APs at the SAN exits emerge from heterogeneous subcellular subthreshold Ca signals, similar to multiscale complex processes of impulse generation within clusters of neurons in neuronal networks (Bychkov et al., 2020;Bychkov et al., 2022). Possible specific mechanisms of signal processing within the network also include stochastic resonance (Clancy and Santana, 2020;Grainger et al., 2021), or a phase transition (Weiss and Qu, 2020;Maltsev et al., 2022a), and electrotonic interactions of firing cells with non-firing cells (Fenske et al., 2020).

Here we tested numerically a hypothesis that a fully functional pacemaker module generating rhythmic APs can be constructed exclusively from loosely connected dormant non-firing cells. Specifically, such module can be constructed with non-excitable cells (Ca oscillators) generating oscillatory local Ca releases driving rhythmic AP firing of an excitable cell (AP generator) lacking intrinsic automaticity. Thus, cardiac impulse can be seen as an emergent property of the SAN cellular network/meshwork and it can be initiated by a community of dormant cells, i.e. without "the pacemaker cell" in its classical definition.

Methods

We used our previously published three-dimensional SAN cell model operating normally as a coupled clock system (Stern et al., 2014): (i) a Ca clock featuring individual stochastically operating RyRs embedded in the cell-distributed Ca store (sarcoplasmic reticulum) equipped with Ca pump and (ii) a membrane clock featuring the full set of electrophysiology equations describing V_m and 10 different ion currents (Maltsev et al., 2022b). The clocks are coupled via

electrogenic Na/Ca exchanger and also via individual L-type Ca channels of two types, Cav1.2 and Cav1.3 differing in their activation voltages by 16 mV, stochastically operating and interacting with RyRs in couplons. We modelled a fully functional pacemaker module as a system of five loosely coupled dormant cells (Fig. 1). Each dormant cell model was a modification of our original model to exclude spontaneous AP firing. Four cells in the pacemaker module were modelled as pure Ca oscillators, having relatively high sarcoplasmic reticulum Ca pumping rate P_{up} and Na/Ca exchange rate, but lacking I_{CaL} to prevent AP generation rendering cells completely both non-excitable and non-firing, but generating subthreshold signals. These four Ca oscillators were connected to one cell that was modelled as a highly excitable cell with large density of Cav1.2 but lacking automaticity in the absence of the low voltage activated Cav1.3. The idea for such functional module was inspired by our recent experimental observation in intact SAN tissue (Bychkov et al., 2022) that some SAN cells generate only oscillatory LCRs that precede AP-induced Ca transients in adjacent cells (Video 2). Thus, in our model the oscillatory subthreshold signals from the four Ca oscillators would be transferred via a low conductance connection to the membrane of the excitable cell, with the cell membrane acting as an integrator (a computer) of the oscillatory signals that depolarize the cell membrane towards the activation I_{CaL} threshold, triggering an AP.

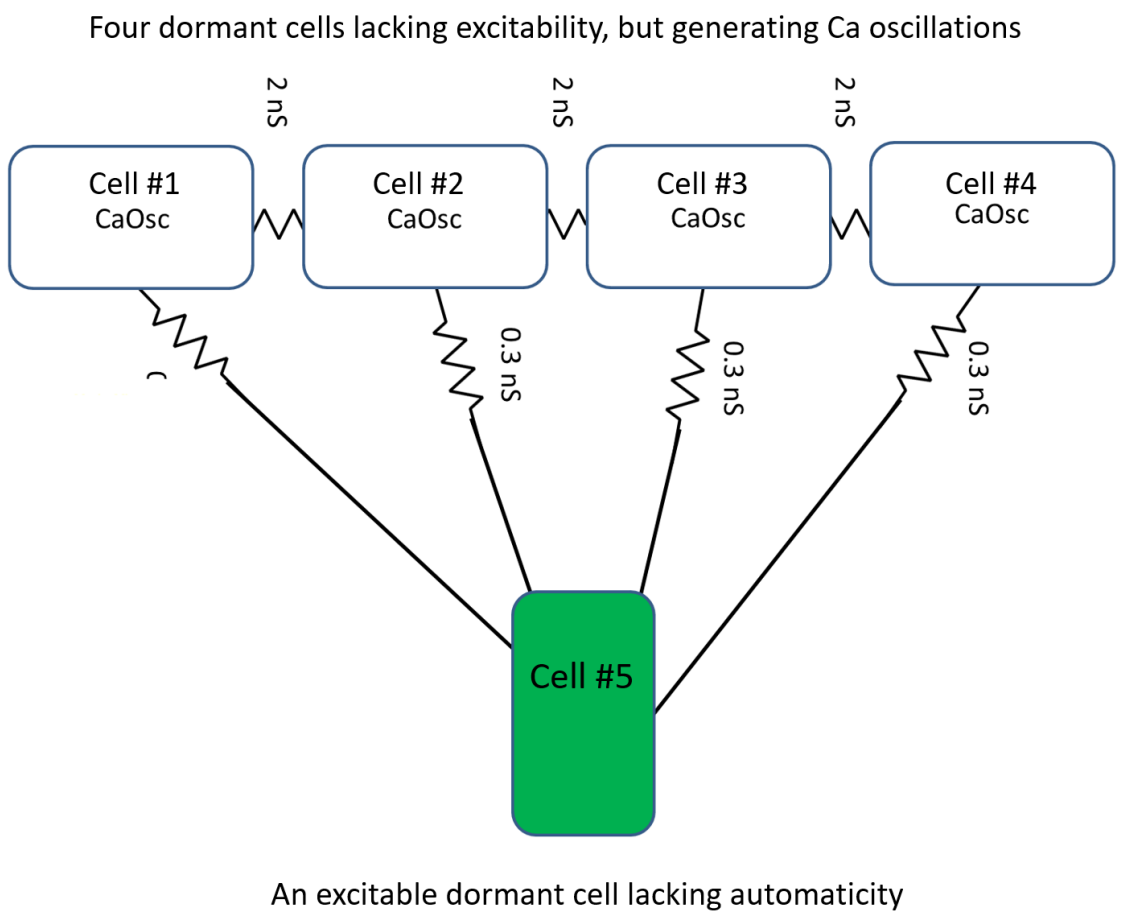

Figure 1. Our prototype of the pacemaker module consisting of dormant cells (lacking intrinsic automaticity): Cells #1-4 are Ca oscillators (CaOsc) and Cell #5 is an excitable cell (AP generator). All cells are loosely connected as shown by zigzag lines with respective conductance (in nS).

Results

First, we simulated each cell model behavior in separation (uncoupled cells) for 2 s. Time series of major cell characteristics are shown in Fig. 2A and B. Cell#1 generated oscillations of submembrane [Ca], NCX current (I_{NCX}), and V_m. Importantly, the V_m oscillations were driven by respective I_{NCX} oscillations and had a relatively small amplitude (vs. an AP). At the same time, cell #5 failed to generate APs, with its V_m fluctuating at a -50 mV level. Then we performed

similar simulation for the coupled-cell system (Fig. 2, panels C, D) with intercellular conductances shown in Fig.1. While the Ca oscillator cells #1-4 continued to generate subthreshold V_m oscillations (basically as in separation), the excitable cell#5 generated rhythmic APs and AP-induced Ca transients paced by cells #1-4. Importantly, the pacing signals in the cell network always preceded each AP generation (inset in Fig.2C and Video 3).

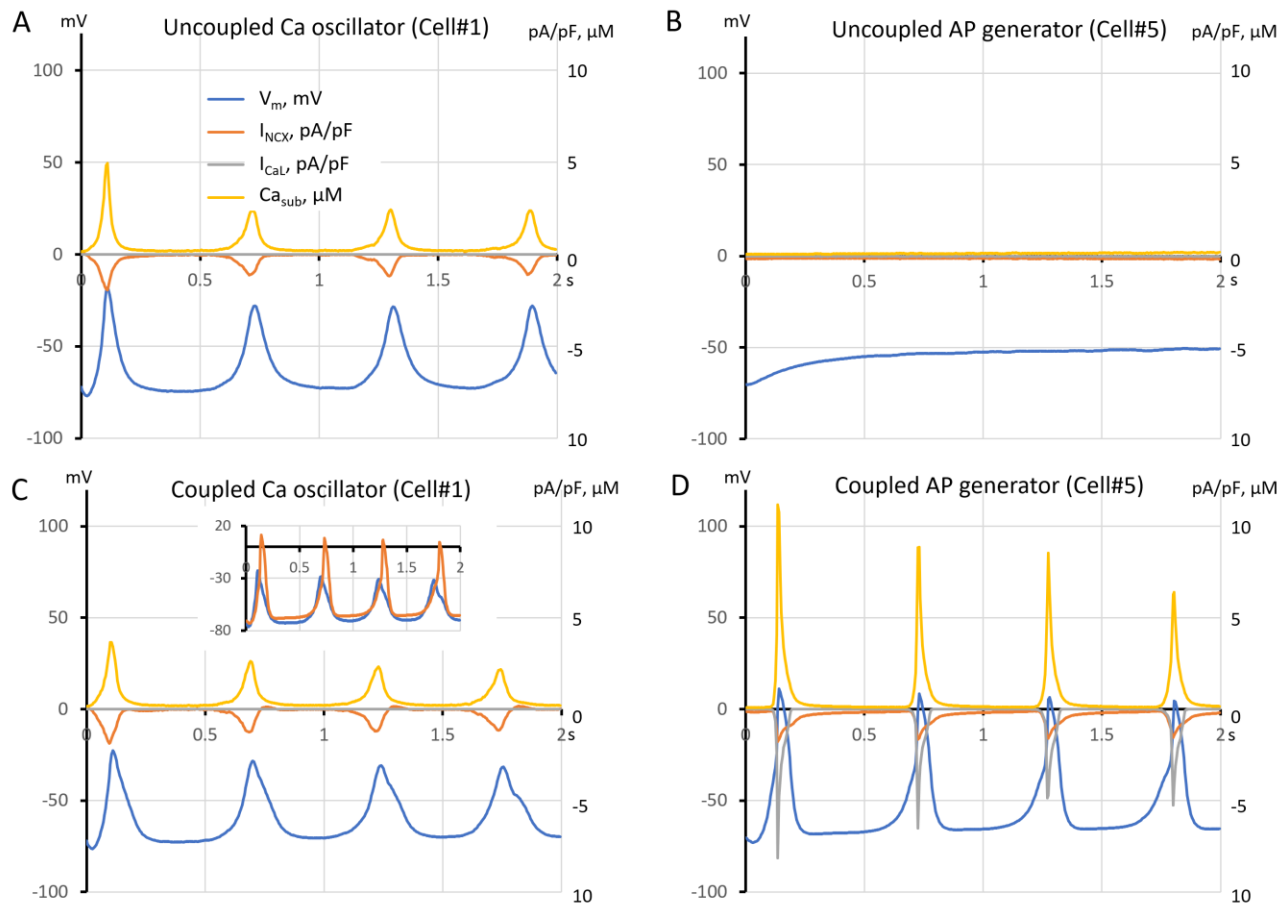

Figure 2. Simulation results: Time series of I_{NCX}, I_{CaL}, V_m, and mean submembrane Ca (Ca_{sub}) simulated for cell #1 (Ca oscillator) and cell #5 (AP generator) in separation (A and B) and when cells were coupled (C and D) as shown in Fig.1. Inset in panel C shows overlapped V_m in coupled cells #1 (blue) and #5 (orange). V_m rise in cell #1 always preceded each AP upstroke in cell#5. See also related Video 3.

Discussion

Here we demonstrate for the first time that rhythmic cardiac pacemaker impulses can be generated by an ensemble of heterogeneous (specialized) dormant SAN cells, i.e. cells lacking intrinsic automaticity. Such dormant cells have been identified in isolation (Kim et al., 2018;Tsutsui et al., 2018;Grainger et al., 2021;Tsutsui et al., 2021;Louradour et al., 2022) and in intact SAN tissue (Bychkov et al., 2020;Fenske et al., 2020). While the function of dormant cells remains unknown, it has been speculated that: 1) dormant cells stay "in reserve" and are "awaken" to function by β-adrenergic stimulation in the fight-or-flight response (Kim et al., 2018;Louradour et al., 2022); 2) non-firing cells can influence the frequency of AP firing cells via electrotonic interactions (Fenske et al., 2020). Experimental studies, however, have shown that dormant cells are not static or neutral: they do generate subthreshold signals (Kim et al., 2018;Bychkov et al., 2020;Grainger et al., 2021). One specific pattern of Ca signaling observed in experimental recordings in intact SAN tissue was that "some SAN cells generate only LCRs that precede AP-induced Ca transients in adjacent cells" (see an example in Video 2). It was then further speculated that such subthreshold signals can somehow spatiotemporally summate and prompt initiation of full-scale signals (i.e. APs) within SAN tissue, e.g. via a stochastic resonance (Clancy and Santana, 2020;Grainger et al., 2021;Guarina et al., 2022), or a phase transition-like process each cycle (Weiss and Qu, 2020;Maltsev et al., 2022a). Thus, following this logic, in addition to simple "wake-up" function under stress or tonic influence, dormant cells can be in fact functionally active (i.e. not fully dormant) generating important subthreshold signals. Here we follow these developments at the frontiers of the pacemaker research and suggest a simple (basic) pacemaker multi-cellular module that can indeed operate via

synchronization and integration of the subthreshold signals in a community of heterogeneous dormant cells that can reach a threshold each cycle to generate rhythmic APs.

The requirement of heterogeneity for such synergistic pacemaker system in our model is in line with that SAN tissue indeed represents an extremely heterogenous structure (Boyett et al., 2000). For example, major ion membrane cell conductances such as of I_{CaL} and I_f vary by an order of magnitude (Honjo et al., 1996;Monfredi et al., 2018). Dormant cells represent a major cell population (>50%) isolated from SAN (Nakayama et al., 1984;Kim et al., 2018;Grainger et al., 2021;Tsutsui et al., 2021;Louradour et al., 2022). Thus, while the existence, properties, and role of the new type of synergistic pacing will need more experimental and theoretical studies, the SAN tissue seems to be perfectly poised to such operation, especially in the basal state firing or under parasympathetic stimulation, i.e. whenever the dormant cells become even more abundant in the SAN.

Another requirement is weak cell coupling. When we connected cells in the model via a stronger conductance (x10), the model failed to generate APs (not shown). Such additional requirement is also well supported by experimental data: the SAN center lacks high conductance CX43 gap junctions (Boyett et al., 2006;Bychkov et al., 2020). The weak coupling allows cells to function independently, i.e. specialize. In our model cells operate as a Ca clock or AP generator. When we artificially increase intercellular conductances, the AP generator gets additional electrotonic load of neighboring cells and its number of Ca channels become insufficient to drive the AP upstroke in the system. In case of perfectly connected cells, the system becomes one giant cell with the respective large net membrane electric capacitance of all cells in the network, but relatively low number of Ca channels, incapable of generating an AP.

What are potential role and benefits of the new pacemaker mechanism of heterogeneous SAN tissue? One could be robust AP firing that keeps the system alive at the edge of criticality (Campana et al., 2022;Maltsev et al., 2022a), i.e. at low heart rates and bradyarrhythmia in aging and disease. Further, such heterogeneous system is expected to save energy: dormant cells, generating subthreshold signals, do not generate high energy-demanding APs. Finally, it is flexible: I_{CaL} and sarcoplasmic reticulum Ca cycling increase during β-adrenergic stimulation and the system operation can quickly change from its energy-saving mode to a highly synchronized AP firing mode, i.e. dormant cells become AP-generating cells featuring classical paradigm of pacemaking (Kim et al., 2018;Tsutsui et al., 2018;Tsutsui et al., 2021;Louradour et al., 2022).

Discussing specific mechanisms of our new network-based pacemaker mechanism, it is important to emphasize the critical role of Ca clock and NCX that appear to be important not only for automaticity of individual SAN cells (Maltsev and Lakatta, 2009;Lyashkov et al., 2018;Yue et al., 2020), but also among the cells withing the SAN tissue: subthreshold Ca signals of synchronized LCRs are translated to respective V_m oscillations via NCX and further summated (i.e. computed) by the membrane of the AP generating cells in the neighborhood. The contributions of Cav1.2 and Cav1.3 are also critically important: the AP generating cell lacking automaticity must have a substantial contribution of high-threshold activation Cav1.2 in order to be highly excitable but at the same time remain dormant. Increasing contribution of low-threshold activation isoform Cav1.3 initiates ignition process that drives automaticity (Torrente et al., 2016;Lyashkov et al., 2018;Maltsev et al., 2022b). Thus, the amplitude of I_{CaL} and the fraction of Cav1.2/Cav1.3 may regulate the occurrence and contribution of different modalities of pacemaking (the new dormant cell-based model vs. classical paradigm).

Pacemaker phase, or slow diastolic depolarization, or phase 4 is thought to be a unique intrinsic property of SAN cells underlying heart automaticity. Our model provides a broader interpretation of this concept: the diastolic depolarization can also emerge as a summation of signals from neighboring cells via their low-conductance interface (Fig. 2D). The low-conductance circuit represents a low-pass filter (with the "RC" time constant of the circuit) and fully transmits slow signals of Ca oscillations to AP-generating cell(s) but filter out transitory signals of the AP upstroke phase in the opposite direction, acting as a diode in electronics or a synaptic-like transition in neurons.

In more general context, the shift of paradigm from pacemaker-cell based to network-based type of pacing is not unique: a similar paradigm shift has happened recently in studies of respiratory rhythm: the respiratory oscillations arise from complex and dynamic molecular, synaptic and neuronal interactions within a diverse neural microcircuit, and "pacemaker properties are not necessary for respiratory rhythmogenesis" (Feldman and Kam, 2015). However, it is important to emphasize that our new dormant cell-based pacemaker mechanism does not refute or substitute the classic pacemaker cell-based mechanism: both mechanisms can co-exist and interact in the form of different functional modules within the central SAN area (Bychkov et al., 2020;Norris and Maltsev, 2022), with the classic mechanism likely prevailing in fight-or-flight response but with dormant cell-based mechanism likely prevailing at low heart rates. Rather, our results challenge the exclusive role of the fully automatic pacemaker cells in the emergent automaticity of SAN, because a fully functional pacemaker module can emerge from subthreshold signal integration (computation) within a dormant cell community.

Acknowledgments

This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

Disclosures

None

Video legends

Video 1

An example of heterogeneous Ca signaling in central mouse SAN that includes rhythmic generation of AP-induced Ca transients in some cells and local subthreshold signals (LCRs) in other cells. Ca recording from (Bychkov et al., 2020).

Video 2

An example of a local Ca signaling module in central mouse SAN in which some SAN cells generate only LCRs that precede AP-induced Ca transients in adjacent cells. Ca recording from (Bychkov et al., 2020). For exact anatomical location within intact SAN and detailed analysis of this video, see Fig. 6 in (Bychkov et al., 2020).

Video 3

Simulation results of our new pacemaker cell-coupled system comprised of dormant cells (Fig. 1). Upper panel: Oscillatory synchronized LCRs under cell membrane in non-excitable Cell #1 (Ca oscillator). Lower panel: AP-induced Ca transients in excitable Cell #5, lacking intrinsic

automaticity but paced by Ca oscillators in cells #1-5. Simulation duration is 2 s (see related time course of Vm, Fig. 2C,D). Note: Ca oscillations in cell #1 always precede AP-induced Ca transients (global Ca spike) in cell #5. The panels are the cell cylinder surfaces unwrapping to squares with distributions of RyR clusters under the cell membrane shown by cyan shades reflecting Ca loading of the junctional sarcoplasmic reticulum in which the clusters are embedded: 0 is pure black and >600 μM is pure cyan. Local Ca dynamics is shown by red shades: 0.1 μM is pure black (resting Ca level) and >5 μM is pure red. Simulations were performed using our modified model of SAN cell in 3 dimensions with individual RyRs and L-type Ca channels (Stern et al., 2014).

References

Arvanitaki, A., Fersard, A., Kruta, A., and Kruta, V. (1937). Reactions electroniques du myocarde en function de sur tonus initial. *Compt. Rend. Sot. Biol.* 124**,** 165-167.

Bleeker, W.K., Mackaay, A.J., Masson-Pevet, M., Bouman, L.N., and Becker, A.E. (1980). Functional and morphological organization of the rabbit sinus node. *Circ Res* 46**,** 11-22.

Boyett, M.R., Honjo, H., and Kodama, I. (2000). The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc Res* 47**,** 658-687.

Boyett, M.R., Inada, S., Yoo, S., Li, J., Liu, J., Tellez, J., Greener, I.D., Honjo, H., Billeter, R., Lei, M., Zhang, H., Efimov, I.R., and Dobrzynski, H. (2006). Connexins in the sinoatrial and atrioventricular nodes. *Adv Cardiol* 42**,** 175-197. doi: 10.1159/000092569.

Bychkov, R., Juhaszova, M., Calvo-Rubio Barrera, M., Donald, L.a.H., Coletta, C., Shumaker, C., Moorman, K., Sirenko, S.T., Maltsev, A.V., Sollott, S.J., and Lakatta, E.G. (2022). The Heart's Pacemaker Mimics Brain Cytoarchitecture and Function: Novel Interstitial Cells Expose Complexity of the SAN. *JACC Clin Electrophysiol*. doi: 10.1016/j.jacep.2022.07.003.

Bychkov, R., Juhaszova, M., Tsutsui, K., Coletta, C., Stern, M.D., Maltsev, V.A., and Lakatta, E.G. (2020). Synchronized cardiac impulses emerge from multi-scale, heterogeneous local calcium signals within and among cells of heart pacemaker tissue. *JACC Clin Electrophysiol* 6**,** 907-931. doi: 10.1016/j.jacep.2020.06.022.

Campana, C.A., Ricci, E., Bartolucci, C., Severi, S., and Sobie, E.A. (2022). Coupling and Heterogeneity Modulate Pacemaking Capability in Healthy and Diseased Two-Dimensional Sinoatrial Node Tissue Models. *BioRxiv* https://doi.org/10.1101/2022.04.14.488275**,** (preprint).

Clancy, C.E., and Santana, L.F. (2020). Evolving Discovery of the Origin of the Heartbeat: A New Perspective on Sinus Rhythm. *JACC Clin Electrophysiol* 6**,** 932-934. doi: 10.1016/j.jacep.2020.07.002.

Efimov, I.R., Nikolski, V.P., and Salama, G. (2004). Optical imaging of the heart. *Circ Res* 95**,** 21-33. doi: 10.1161/01.RES.0000130529.18016.35.

Feldman, J.L., and Kam, K. (2015). Facing the challenge of mammalian neural microcircuits: taking a few breaths may help. *J Physiol* 593**,** 3-23. doi: 10.1113/jphysiol.2014.277632.

Fenske, S., Hennis, K., Rotzer, R.D., Brox, V.F., Becirovic, E., Scharr, A., Gruner, C., Ziegler, T., Mehlfeld, V., Brennan, J., Efimov, I.R., Pauza, A.G., Moser, M., Wotjak, C.T., Kupatt, C., Gonner, R., Zhang, R., Zhang, H., Zong, X., Biel, M., and Wahl-Schott, C. (2020). cAMP-dependent regulation of HCN4 controls the tonic entrainment process in sinoatrial node pacemaker cells. *Nat Commun* 11**,** 5555. doi: 10.1038/s41467-020-19304-9.

Grainger, N., Guarina, L., Cudmore, R.H., and Santana, L.F. (2021). The Organization of the Sinoatrial Node Microvasculature Varies Regionally to Match Local Myocyte Excitability. *Function (Oxf)* 2**,** zqab031. doi: 10.1093/function/zqab031.

Guarina, L., Moghbel, A.N., Pourhosseinzadeh, M.S., Cudmore, R.H., Sato, D., Clancy, C.E., and Santana, L.F. (2022). Biological noise is a key determinant of the reproducibility and adaptability of cardiac pacemaking and EC coupling. *J Gen Physiol* 154. doi: 10.1085/jgp.202012613.

Honjo, H., Boyett, M.R., Kodama, I., and Toyama, J. (1996). Correlation between electrical activity and the size of rabbit sino-atrial node cells. *J Physiol* 496 (Pt 3)**,** 795-808.

Jalife, J. (1984). Mutual entrainment and electrical coupling as mechanisms for synchronous firing of rabbit sino-atrial pace-maker cells. *J Physiol* 356**,** 221-243.

Keith, A., and Flack, M. (1907). The Form and Nature of the Muscular Connections between the Primary Divisions of the Vertebrate Heart. *J Anat Physiol* 41**,** 172-189.

Kim, M.S., Maltsev, A.V., Monfredi, O., Maltseva, L.A., Wirth, A., Florio, M.C., Tsutsui, K., Riordon, D.R., Parsons, S.P., Tagirova, S., Ziman, B.D., Stern, M.D., Lakatta, E.G., and Maltsev, V.A. (2018). Heterogeneity of calcium clock functions in dormant, dysrhythmically and rhythmically firing single pacemaker cells isolated from SA node. *Cell Calcium* 74**,** 168-179. doi: 10.1016/j.ceca.2018.07.002.

Lang, D., Petrov, V., Lou, Q., Osipov, G., and Efimov, I.R. (2011). Spatiotemporal control of heart rate in a rabbit heart. *J Electrocardiol* 44**,** 626-634.

Li, N., Hansen, B.J., Csepe, T.A., Zhao, J., Ignozzi, A.J., Sul, L.V., Zakharkin, S.O., Kalyanasundaram, A., Davis, J.P., Biesiadecki, B.J., Kilic, A., Janssen, P.M.L., Mohler, P.J., Weiss, R., Hummel, J.D., and Fedorov, V.V. (2017). Redundant and diverse intranodal pacemakers and conduction pathways protect the human sinoatrial node from failure. *Sci Transl Med* 9. doi: 10.1126/scitranslmed.aam5607.

Louradour, J., Bortolotti, O., Torre, E., Bidaud, I., Lamb, N., Fernandez, A., Le Guennec, J.Y., Mangoni, M.E., and Mesirca, P. (2022). L-Type Cav1.3 Calcium Channels Are Required for Beta-Adrenergic Triggered Automaticity in Dormant Mouse Sinoatrial Pacemaker Cells. *Cells* 11. doi: 10.3390/cells11071114.

Lyashkov, A.E., Behar, J., Lakatta, E.G., Yaniv, Y., and Maltsev, V.A. (2018). Positive Feedback Mechanisms among Local Ca Releases, NCX, and ICaL Ignite Pacemaker Action Potentials. *Biophys J* 114**,** 1176-1189. doi: 10.1016/j.bpj.2017.12.043.

Maltsev, A.V., Stern, M.D., Lakatta, E.G., and Maltsev, V.A. (2022a). Functional heterogeneity of cell populations increases robustness of pacemaker function in a numerical model of the sinoatrial node tissue. *Front Physiol* 13**,** 845634. doi: 10.3389/fphys.2022.845634.

Maltsev, A.V., Stern, M.D., and Maltsev, V.A. (2022b). Disorder in Ca2+ release unit locations confers robustness but cuts flexibility of heart pacemaking. *J Gen Physiol* 154. doi: 10.1085/jgp.202113061.

Maltsev, V.A., and Lakatta, E.G. (2009). Synergism of coupled subsarcolemmal Ca^{2+} clocks and sarcolemmal voltage clocks confers robust and flexible pacemaker function in a novel pacemaker cell model. *Am J Physiol Heart Circ Physiol* 296**,** H594-H615.

Michaels, D.C., Matyas, E.P., and Jalife, J. (1987). Mechanisms of sinoatrial pacemaker synchronization: a new hypothesis. *Circ Res* 61**,** 704-714.

Monfredi, O., Tsutsui, K., Ziman, B., Stern, M.D., Lakatta, E.G., and Maltsev, V.A. (2018). Electrophysiological heterogeneity of pacemaker cells in the rabbit intercaval region, including the SA node: insights from recording multiple ion currents in each cell. *Am J Physiol Heart Circ Physiol* 314**,** H403-H414. doi: 10.1152/ajpheart.00253.2016.

Nakayama, T., Kurachi, Y., Noma, A., and Irisawa, H. (1984). Action potential and membrane currents of single pacemaker cells of the rabbit heart. *Pflugers Arch* 402**,** 248-257.

Noble, D. (1962). A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pace-maker potentials. *J Physiol* 160**,** 317-352.

Norris, C.F., and Maltsev, A.V. (2022). Meaningful Local Signaling in Sinoatrial Node Identified by Random Matrix Theory. *BioRxiv, https://doi.org/10.1101/2022.02.25.482007 (preprint).*

Sano, T., Sawanobori, T., and Adaniya, H. (1978). Mechanism of rhythm determination among pacemaker cells of the mammalian sinus node. *Am J Physiol* 235**,** H379-384. doi: 10.1152/ajpheart.1978.235.4.H379.

Stern, M.D., Maltseva, L.A., Juhaszova, M., Sollott, S.J., Lakatta, E.G., and Maltsev, V.A. (2014). Hierarchical clustering of ryanodine receptors enables emergence of a calcium clock in sinoatrial node cells. *J Gen Physiol* 143**,** 577-604.

Torrente, A.G., Mesirca, P., Neco, P., Rizzetto, R., Dubel, S., Barrere, C., Sinegger-Brauns, M., Striessnig, J., Richard, S., Nargeot, J., Gomez, A.M., and Mangoni, M.E. (2016). L-type Cav1.3 channels regulate ryanodine receptor-dependent Ca^{2+} release during sino-atrial node pacemaker activity. *Cardiovasc Res* 109**,** 451-461. doi: 10.1093/cvr/cvw006.

Tsutsui, K., Florio, M.C., Yang, A., Wirth, A.N., Yang, D., Kim, M.S., Ziman, B.D., Bychkov, R., Monfredi, O.J., Maltsev, V.A., and Lakatta, E.G. (2021). cAMP-Dependent Signaling Restores AP Firing in Dormant SA Node Cells via Enhancement of Surface Membrane Currents and Calcium Coupling. *Front Physiol* 12**,** 596832. doi: 10.3389/fphys.2021.596832.

Tsutsui, K., Monfredi, O., Sirenko-Tagirova, S.G., Maltseva, L.A., Bychkov, R., Kim, M.S., Ziman, B.D., Tarasov, K.V., Tarasova, Y.S., Zhang, J., Wang, M., Maltsev, A.V., Brennan, J.A., Efimov, I.R., Stern, M.D., Maltsev, V.A., and Lakatta, E.G. (2018). A coupled-clock system drives the automaticity of human sinoatrial nodal pacemaker cells. *Sci Signal* 11**,** eaap7608.

Weiss, J.N., and Qu, Z. (2020). The Sinus Node: Still Mysterious After All These Years. *JACC Clin Electrophysiol* 6**,** 1841-1843. doi: 10.1016/j.jacep.2020.09.017.

Yue, X., Hazan, A., Lotteau, S., Zhang, R., Torrente, A.G., Philipson, K.D., Ottolia, M., and Goldhaber, J.I. (2020). Na/Ca exchange in the atrium: Role in sinoatrial node pacemaking

and excitation-contraction coupling. *Cell Calcium* 87**,** 102167. doi: 10.1016/j.ceca.2020.102167.