Dormant SANC (ones that don't fire spontaneous action potentials (APs)) may therefore represent the extreme of clock uncoupling. We hypothesized that increased cAMP-mediated, PKA-dependent phosphorylation of the coupled-clock system proteins would restore rhythmic AP firing in dormant SANC. We recorded membrane potential (Vm) and intracellular Ca^{2+} kinetics in enzymatically isolated single, dormant guinea pig SANC prior to and during β-adrenoceptor stimulation (BARs, isoproterenol 0.1-1 μM) or during exposure to cell-permeant cAMP (CPT-cAMP, 300 μM). Dormant SANC that did not fire still maintained a resting membrane potential of −35+/-3mV (n=17), and exhibited small, random LCRs. ~40% responded to BARs/CPT-cAMP. This initial response involved increases in LCR size and rhythmicity, and hyperpolarization of Vm, accompanied by small amplitude, spontaneous APs (rate ~1Hz) triggering whole-cell Ca^{2+} transients. During the subsequent transition to steady-state AP firing, average LCR size increased while average LCR period shortened, partially synchronizing LCRs to late diastole, (rate ~2Hz); this was paralleled by MDP hyperpolarization to ~-60 mV. AP amplitude increased, and average AP cycle length shortened similar to LCR period. When BARs/CPT-cAMP was washed out, all changes reversed in order, and SANC again exhibited small random LCRs, with Vm ~-40 mV. In conclusion, at least in some SANC, dormancy represents membrane & Ca^{2+} clock uncoupling that is rescuable through increased cAMP and PKA-dependent phosphorylation of both membrane and Ca^{2+} clock proteins. Specifically, rescuing impaired clock coupling occurs through: (1) increased **spatial** LCR synchronization, yielding larger LCRs; (2) increased **temporal** LCR synchronization, decreasing average LCR period and shifting larger LCR occurrence to late diastole.

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A Sexy Approach to Pacemaking

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Introduction: The sinoatrial node (SAN) is the primary pacemaker of the heart. Clinical evidence shows that SAN function differs between sexes. Males have slower resting (extrinsic and intrinsic) heart rates, and longer corrected SAN recovery time compared with females. **Hypothesis:** Sex based differences may be due to SAN ion channel differences. Therefore, we investigated functional behaviour and molecular makeup of SANs in male and female rats. **Methods**: Intracellular action potential recordings were obtained using sharp microelectrode technique (n=5 each). Gene expression was measured with quantitative PCR (n=6 each). **Results**: The amplitude of the action potential was significantly higher in male SANs (45.3 ± 2.4) compared with female (31.7 ± 2.5). The upstroke velocity (dV/dt_{max}) was significantly faster in female SANs (16.4 ± 1.6) compared with male (9.1 ± 1.5). Out of 96 transcripts investigated, two were significantly different between the sexes. L-Type Ca^{2+} channel, Cav1.3, and acetylcholine activated K+ channel, Kir3.1, mRNA levels were significantly higher in female SANs (66.6% and 45.6% respectively) compared with male. Immunolabelling for $Ca_v1.3$ showed levels were significantly higher in female SANs compared with male (18.6%, n=6 each). Inhibition of Cav1.3 with nifedipine (5μM) showed greater effect on the beating rate of male SANs compared with female (43%). A non-coding RNA, miR-139-3p (predicted by Ingenuity IPA software to affect Cav1.3) showed greater effect on the beating rate of male SAN preparations compared with female. Mathematical modelling showed that a higher density of Cav1.3 (as in the case of females) has a protective role from sinoatrial node arrhythmias. **Conclusion**: This study identified functional and molecular differences in key pacemaker ion channels in SAN between sexes. These differences advance our understanding of gender differences in cardiac electrophysiology and may have implications for gender specific design of anti-arrhythmic drugs and biological pacemakers.

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A Methodology to Improve Human Ventricular Models for the Investigation of Cardiac Arrhythmias

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Introduction: A number of human action potential (AP) models can be found in the literature for the study of ventricular arrhythmias. One of the latest proposed models is the Carro-Rodríguez-Laguna-Pueyo (CRLP). Although this model reproduces many human ventricular physiological features, it lacks the characteristic AP peak and dome. In this work we propose a modification of the L-type Calcium current (I_{CaL}) to improve its AP shape and to best fit the model to experimental results.
Methods: Both the time constants and the steady-state value of the I_{CaL} inactivation gates were modified, with validation performed at two different levels: 1)The results for the new I_{CaL} formulation were compared against three different experimental datasets using in silico simulations of the experimental protocols. 2)A set of arrhythmic risk markers were calculated using the modified AP model, including: systolic and diastolic intracellular calcium concentration ($[Ca^{2+}]_i$), AP duration, AP triangulation at different pacing frequencies, and APD adaptation to abrupt cycle length changes.
Results: The mean square error of steady-state I_{CaL} inactivation was 25% lower than in the original CRLP model and satisfactorily reproduced the available experimental data. The modified time constants of the I_{CaL} inactivation gates led to physiological AP peak and dome shapes. All the computed arrhythmic risk markers remained in the physiological range or were very similar to the ones reported for the original CRLP model.
Conclusions: The proposed modification in the CRLP model improves the behavior of the I_{CaL} current and the AP shape, providing a model suitable for the investigation of ventricular arrhythmias. The strategy proposed in this work can be extended to other human ventricular cell models available in the literature.

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Memory and Stability of the Cardiac Action Potential Repolarization in the Space of its Allowed States: A Single Cell Simulation Study

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The rate-dependent partition of the cardiac cycle into diastole and systole is measured at the cellular level as rate-dependence (RD) and electrical restitution (ER) of the action potential (AP) duration (APD), the first describing changes in APD after steady state changes in cycle length (CL), and the second APD changes after a sudden switch from constant to a variably delayed CL, as a function of the pre-switch CL (or DI). A dynamic ER curve (DER) can also be measured under beat-to-beat changes in pacing rate, and the increase in its slope is a recognized predictor of electrical alternance and ventricular fibrillation. An hysteretic behavior has been described previously in DER curve and associated with cardiac memory and repolarization stability. In the present study, by means of simulations with three numerical models of the human ventricular AP, I measure DER as APD vs DI and APD vs CL plots from high frequency paced AP series, and find that both show hysteresis with remarkably different phase shifts. Based on both representations, I define and measure a space of states (SPoS) for APs elicited, in turn, at constant, periodically changing, and randomly changing (1, 2, and 3 dimensional SPoS) pacing rates, and use them to study perturbations (a single missing beat) of the pacing protocol. I show how the maximum conductance and gating kinetics of three plateau ion currents (I_{Ks}, I_{Kr}, and I_{CaL}), change shape and size of SPoS. I show how geometrical parameters of SPoS, for a given AP type and a given pacing law, can be used to numerically quantify memory and stability of its dynamics.

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An Integrated Model of Human Beta-Adrenergic Signaling and Ventricular Electrophysiology Reveals Contributors to Positive Inotropy

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Adrenergic regulation is well recognized as an important player in cardiac functions and contributes to pathology underlying arrhythmia and heart failure. Mathematical models of beta-adrenergic signaling have been developed and adrenergic effects on cardiac electrophysiology have been analyzed in animal cells. However, key contributors of these effects in human myocytes remain largely unknown. We have integrated detailed beta-adrenergic signaling cascade with human ventricular electrophysiology based on published models of these two systems. PKA targets in human ventricular electrophysiology: fast sodium channel, slow delayed rectifier potassium channel, sodium potassium ATPase, L-type calcium channel, ryanodine receptor, phospholamban and troponin-I, are simulated to incorporate both baseline and phosphorylation-modified behaviors. Responses to different concentrations of adrenergic agonist isoproterenol are also monitored and adjusted. The model was validated by ensuring these behaviors matched published voltage-clamp data before and after phosphorylation. Simulations to analyze sensitivity of each PKA target were performed with blocking the phosphorylation of a single target one at a time. These simulations showed that phosphorylation of L-type calcium channel and phospholamban were by far the largest contributors to the positive