Iso+BayK-induced EADs by fast pacing rates and Ca accumulation; 2) The upstrokes of H_2O_2-induced EADs preceded the initiations of Ca aftertransients (CaATs), while the upstrokes of Iso+BayK-induced EADs occurred after the initiations of CaATs; 3) The EAD take-off potentials were within a narrow range between +20 and −20 mV in H_2O_2 model, while they were randomly distributed in a wide range between +30 and −60 mV, corresponding to concomitant Ca waves in Iso+BayK model; 4) When the cell membrane clamp was switched from current clamp to voltage clamp using a normal AP waveform without EAD, CaATs/Ca waves disappeared in H_2O_2 model, but persisted in Iso+BayK model. 5) The $I_{Ca,L}$ blocker nifedipine (10 μM), eliminated EADs and CaATs in both models. 6) SEA400 (2 μM), a selective blocker of Na-Ca exchange current, suppressed EADs in both models and H_2O_2-inducd CaAT, but exerted less effect on Ca wave in Iso+BayK model. We conclude that the mechanisms for EADs vary depending on their causative factors. While reactivation of $I_{Ca,L}$ plays a predominant role in EAD genesis by H_2O_2, spontaneous Ca waves are predominant cause under Ca-overload conditions (e.g. in Iso+BayK model).

2367-Pos Board B353
Ca^{2+}-Regulated-cAMP/PKA Signaling in Cardiac Pacemaker Cells Links ATP Supply to Demand
Yael Yaniv, Magdalena Juhaszova, Alexey E. Lyashkov, Harold A. Spurgeon, Steven J. Sollott, Edward G. Lakatta.
Rationale: In sinoatrial node cells (SANC), Ca^{2+} activates adenylate cyclase (AC) to generate a high basal level of cAMP-mediated/protein kinase A (PKA)-dependent phosphorylation of Ca^{2+} cycling proteins. This results in spontaneous sarcoplasmic reticulum-generated rhythmic Ca^{2+} oscillations during diastolic depolarization, that not only ignite the surface membrane to generate rhythmic action potentials (APs), but, in a feed-forward manner, also activate AC/PKA signaling. ATP is consumed to produce cAMP, to pump Ca^{2+} and to contract.
Objective: Since nature efficiently links ATP-demand to ATP production, we hypothesized that (1) both basal ATP supply and demand in SANC would be Ca^{2+}-cAMP/PKA dependent; and (2) due to its feed-forward nature, a decrease in flux through the Ca^{2+}-cAMP/PKA signaling axis will reduce the basal ATP level.
Methods and Results: Graded reduction of basal Ca^{2+}-cAMP/PKA signaling in rabbit SANC, produced graded ATP depletion (r^2=0.96), and reduced O_2 consumption and flavoprotein fluorescence. Neither inhibition of glycolysis, nor selectively blocking contraction reduced the ATP level. Specific inhibition of mitochondrial Ca^{2+} flux was without effect, indicating that the cAMP/PKA component rather than Ca^{2+} directly, links the Ca^{2+}/cAMP-PKA signaling to ATP production.
Conclusions: Feed-forward basal Ca^{2+}-cAMP/PKA signaling both consumes ATP to drive spontaneous APs in SANC and is tightly linked to mitochondrial ATP production. Interfering with Ca^{2+}-cAMP/PKA signaling to reduce the SANC ATP demand also "pulls the plug" on SANC ATP supply. This distinctly differs from ventricular myocytes, which lack this feed-forward basal cAMP/PKA signaling, and in which ATP level remains constant when the demand changes.

2368-Pos Board B354
di-4-ANEPPS Slows Cardiac Conduction Velocity
Katie J. Sciuto, Anders Peter Larsen, Alonso P. Moreno, Steven Poelzing.
One of the most widely used voltage-sensitive dyes for optically mapping cardiac conduction is di-4-ANEPPS. Previous studies suggest that di-4-ANEPPS broadens the QRS; however, little is known about its effects on myocardial conduction. We hypothesized that di-4-ANEPPS suppresses cardiac conduction velocity (CV).
CV was quantified in Langendorff-perfused guinea pig hearts using unipolar electrode and optical recordings. Electrode recordings from the anterior epicardium revealed that di-4-ANEPPS (7.5 μM) slowed cardiac transverse CV significantly from 23 ± 4 cm/s to 18 ± 3 cm/s ($p<0.05$). To investigate a possible concentration dependent effect of di-4-ANEPPS, CV and anisotropy was quantified using optical signals recorded from the anterior epicardium of both the right and left ventricle (RV, LV) at different concentrations of di-4-ANEPPS. Increasing the concentration of di-4-ANEPPS from 1.9 to 15 μM reduced transverse CV by 7 ± 2 cm/s in the RV ($p<0.05$) (n=4) and 4 ± 2 cm/s in the LV ($p<0.05$) (n=4). The decrease in longitudinal CV trended towards significance in both the RV (14 ± 7 cm/s, p=0.08) and the LV (15 ± 9 cm/s, p=0.07). The anisotropic ratio of CV was not affected by di-4-ANEPPS concentration. Connexin43 conductance was not significantly changed by di-4-ANEPPS at 15 μM evident from dual patch clamp experiments on HeLa cell-pairs overexpressing rat connexin43 (n=5), suggesting that decreased gap junction conductance is not the underlying mechanism. These data suggest that the perfusion of di-4-ANEPPS into whole heart tissue slows CV in both the right and left ventricles and this effect does not appear to be connexin related. Investigators should take the effect of di-4-ANEPPS on conduction into account when interpreting data obtained with this dye.

2369-Pos Board B355
Decomposition of Fluoresced Transmembrane Potentials Using Multiresolution Wavelet Analysis
Huda Asfour, Luther Swift, Narine Sarvazyan, Milos Doroslovacki, Matthew Kay.
Fluorescence imaging of transmembrane voltage- sensitive dyes is used to study electrical activation in cardiac tissue. However, fluorescence signals suffer from sensitivity to motion due to loss of registration between the target and the imaging device. We introduce a new processing approach for fluoresced transmembrane potentials (fTmps) that is based upon the discreet wavelet transform. We show how fTmp signals can be decomposed and reconstructed to form three sub-signals that contain signal noise (noise signal), the early depolarization phase of the action potential (rTmp signal), and motion artifact (rMA signal). Rat hearts stained with RH237 were used to obtain fTmp signals contaminated with motion artifact. fTmp signals acquired from the epicardial surface of the heart were decomposed and reconstructed using coiflet4 wavelet. Results indicate that the approach is a useful processing step to remove baseline drift, reduce noise, and reveal wave fronts. In addition, local motion artifact amplitudes can be measured using rMA signals. Multiresolution wavelet analysis can be used to study wave fronts without aggressive mechanical tissue constraint or electromechanical uncoupling agents and is particularly useful for single camera systems that do not provide for ratiometric imaging.

2370-Pos Board B356
Irradiation Leads to Disturbed Excitation-Contraction Coupling in Cardiac Myocytes Through ROS-Dependent CaMKII Activation
Kay Neumann, Can Sag, Hendrik Wolff, Marie Opiela, Hans Christiansen, Lars Maier.
Introduction: Chest radiotherapy is part of therapeutic concepts for malignant diseases. However, radiation-induced cardiotoxic effects have become clinically relevant. The underlying pathomechanisms are poorly understood.
Hypothesis: We investigated whether excitation-contraction coupling in cardiac myocytes, which depends on intact intracellular Ca cycling, may be *directly* affected by irradiation (IR).
Methods: Isolated ventricular mouse myocytes were exposed to graded IR (0 Gy sham IR, 4 Gy and 20 Gy). Ca and Na handling properties and intracellular reactive oxygen species (ROS) levels were measured using epifluorescence microscopy (Fura-2, SBFI and CMH_2-DCFDA, respectively) and confocal Ca (Fluo-4) imaging. Trypan blue staining indicated cellular injury. Western Blots revealed protein phosphorylation levels.
Results: IR increased systolic sarcoplasmic reticulum (SR) Ca release leading to an acute positive-inotropic effect with Ca transients of 0.21 ± 0.01 ratio units (r.u., F340nm/F380nm) at 20 Gy (N=89) vs. 0.15 ± 0.01 r.u. in untreated sham control myocytes (N=98, $P<0.05$ using one-way ANOVA). Although SR Ca content was unaltered, an increased diastolic SR Ca leak measured as Ca sparks (752 ± 113 Ca sparks*pL^{-1}*s^{-1} at 20 Gy, N=32 vs. 208 ± 34 sparks*pL^{-1}*s^{-1} at 0 Gy, N=12, $P<0.05$ using one-way ANOVA) was accompanied by diastolic Ca overload and increased cellular injury. Furthermore, a rise in intracellular Na from 0.76 ± 0.01 r.u. (N=27) at 0 Gy to 0.79 ± 0.01 (N=39, $P<0.05$ using one-way ANOVA) at 20 Gy was observed after IR.
IR-dependent elevation of ROS levels (by ~667% at 20 Gy) contributed to Ca/calmodulin-dependent protein kinase II (CaMKII) activation. CaMKII-inhibition and ROS-scavenging prevented IR-dependent Ca overload, cellular dysfunction, and cell death.
Conclusions: IR severely disturbs cardiac Ca handling and decreases myocyte viability. As underlying pathomechanism, a ROS/CaMKII signaling pathway was identified.

2371-Pos Board B357
Role of Gap Junction Channels on Cardiac Memory
J. Krishnan, V.S Chakravarthy.
The phenomenon of cardiac memory refers to persistent response of the heart to an external pacing stimulus, where the response typically outlasts the stimulus. It is a fundamental tenet of neuroscience that synaptic modification is the basis of various forms of learning and memory. Drawing analogy with neural synapses and Gap Junctions (GJs) in the cardiac tissue, we had earlier hypothesized that dynamic, voltage-sensitive change in GJ conductance may be related to the phenomenon of cardiac memory. In this work we have coupled cardiac cells by GJ conductance. The GJ conductance is allowed to vary as a function of junctional voltage. Simulations show the cell pair and GJ system has two stable states: one at a high value of GJ conductance and another at a lower value. The system can be switched between the two states by an appropriate external input. Such bistable dynamics can support memory operations and most probably underlie the phenomenon of cardiac memory. There are three kinds of cell