anticipate that myocyte-specific VSFP-based voltage imaging will facilitate studies of cardiomyocyte and whole-heart functionality under minimally invasive conditions.

3117-Pos Board B222
Sinoatrial Nodal (SAN) Cells from Center or Peripheral SAN Area are not Functionally Different
Dongmei Yang, Ihor Zahanich, Alexey E. Lyashkov, Edward G. Lakatta.
SAN cells (SANC) determine the rate and rhythmicity of action potentials (AP) that emanate from the SAN to drive the heartbeat. Perspectives gleaned from SAN tissue have been interpreted to indicate that cells from the central SAN, i.e. smaller cells, control the SAN AP firing rate, while data from both SAN and isolated SANC argue that calcium cycling protein density is independent of SAN area and SANC size, and that spontaneous AP cycle length is independent of cell size. Since it is well documented that the gap junction protein, Connexin 43 (Cx43), is largely expressed in the peripheral vs. central SAN areas, we measured the properties of single SANC, employing Cx43 immunolabeling to distinguish cells isolated from the central (Cx43-negative) or peripheral (Cx43-positive) SAN areas.
Freshly isolated adult rabbit SANC from the central area (Cx43-negative) are, on average, smaller ($612.9 \pm 2.5 \mu m^2$, n=340) than peripheral SANC ($818.6 \pm 23.7 \mu m^2$, n=188, $p<0.001$), but there is no difference in the spontaneous AP firing rate (3.07 ± 0.13Hz, n=13, for Cx43-negative and 3.28 ± 0.12Hz, n=23, for Cx43-positive). The AP amplitude and Maximum Diastolic Depolarization also did not differ, but compared to Cx43-positive SANC, the AP of Cx43-negative SANC has a slower AP upstroke (dV/dt_{max} (V/S): 8.12 ± 1.31 *vs.* 13.4 ± 0.62, $p<0.001$) and a longer repolarization time (APD75 (ms): 120.7 ± 4.9 vs. 102.4 ± 2.9, $p<0.01$). Linear regression analyses failed to detect any significant correlations between any AP parameter and the cell size for both. Preliminary data does not show significant differences between Cx43-positive or negative SANC in the AP triggered global Ca^{2+}-transient or spontaneous diastolic Local Ca^{2+} Releases. Our results indicate that although different in size, single isolated central and peripheral SANC, in the basal state, are not functionally different from each other in respect to average spontaneous AP cycle length.

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Cellular Basis of Phase 2 in Mouse Ventricular Action Potentials
Marcela Ferreiro, Ariel L. Escobar.
Ventricular action potentials (AP) are characterized by a fast depolarizing phase followed by a second upstroke known as phase 2 (Ph2). However, mouse cardiac AP is well known for a lack of Ph2. Recently, we have shown that epicardial AP measured with a large diameter optical-fiber Pulsed Local Field Fluorescence Microscope (PLFFM) display a prominent Ph2. The aim of this work is to understand why most of published electrophysiological studies did not show Ph2. Furthermore, we evaluated several hypotheses to explain these paradoxical differences. First, we evaluated if electrical and optical recordings in different regions of the epicardial layer could differentially display Ph2. We performed simultaneous and colocalized recordings by means of PLFFM and intracellular microelectrodes in different regions of the epicardial layer. Although we found that the contribution of Ph2 to the action potential was dissimilar for different positions all of them show a noticeable Ph2 in both the optical and the electrical recordings. Second, we tested if different pharmacological and physiological intervention that on other mammal hearts modifies Ph2 were able to change this late depolarizing component in mice. Interestingly, Nifedipine and Ryanodine can significantly reduce Ph2. Moreover, when Langenforff perfused hearts were cooled down to 22°C, Ph2 was dramatically reduced. Finally, we tested if Ph2 was an emergent property of the tissue and not a characteristic of a single ventricular myocyte. Surprisingly, ventricular myocytes current clamped with sharp microelectrodes display a large Ph2 (71 ± 3 % of the total AP). This Ph2 was significantly decreased at 22°C and when the membrane potential was hyperpolarize to −100 mV. We conclude that mouse ventricular APs display Ph2 under physiological conditions and changes in the intracellular Ca^{2+} dynamics and thermodynamic parameters can abolish Ph2. Supported by NIH-R01-HL-084487 to AE.

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Comparison of Action Potential Characteristics from Intact Rabbit Myocardium Using 2-Photon Excitation, Widefield Epifluorescence and Microelectrode Recordings
Allen Kelly, Ole J. Kemi, Iffath A. Ghouri, Francis L. Burton, Rachel C. Myles, Godfrey L. Smith.
2-photon excitation of voltage-sensitive dyes enables determination of sub-cellular electrical activity within intact myocardium at a range of depths. The investigation aimed to compare action potential (AP) characteristics derived from sub-cellular 2-photon imaging in an intact cardiac preparation using Di-4-ANEPPS with epifluorescence and microelectrode voltage recordings. Hearts from male New Zealand white rabbits were Langendorff-perfused at 37°C and paced at a cycle length of 350ms. Preparations were loaded with Di-4-ANEPPS and optical APs recorded using widefield epifluorescence and 2-photon (2P) microscopy. BDM (10mM) and Blebbistatin (10μM) were used to eliminate motion artefacts. Membrane potentials were recorded at 26KHz before and after perfusion of mechanical uncouplers.
Using surface microelectrodes V_{max} values of up to 120 $V.s^{-1}$ were recorded. Mean 10-90% AP rise times were 2.78 ± 0.29 ms (mean ± S.E.M) using microelectrodes (n=3), compared with 3.91 ± 0.30 ms recorded at 50 μm below tissue surface with 2P imaging (at 2.6KHz). Matching sampling frequency between microelectrode and 2P recordings abolished this difference. Perfusion of mechanical uncouplers did not significantly alter rise time (2.44 ± 0.20 ms *vs.* 2.78 ± 0.29 ms; before *vs.* after, $P=0.4$) or APD_{90} (134.90 ± 4.13 ms *vs.* 130.25 ± 2.93 ms; before *vs.* after, $P=0.4$). Mean AP rise times for 2P recordings lengthened with increasing tissue depth (3.91 ± 0.30 ms *vs.* 5.35 ± 0.23 ms; 50 *vs.* 250μm from surface, $P<0.05$, n=7) while epifluorescence rise times were consistently longer (7.85 ± 0.32 ms, n=7). Composite images from multiple depths using 2P microscopy displayed rise times approaching those recorded from widefield epifluorescence. These data suggest that tissue light-scattering at increasing depths results in lengthening of measured AP rise times without significantly altering APD. Slower rise times combined with more diffuse images obtained at depths greater than 200μm suggests optical aberrations cause loss of signal resolution at these depths.

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Cardiac Vortex Dynamics: From Cell to Tissue
Ashley E. Raba, Jacques Beaumont.
We address issues related to the construction of multiscale models used to investigate the molecular mechanisms of bioelectric disorders.
We construct a channel gating model of the sodium current from simulated data generated with the Ebihara and Johnson model. Three cases are considered, the simulated data set is: complete and noiseless, complete and noisy, and incomplete. The gating model parameters are obtained with an inversion methodology which allows to: determine, *a-priori*, whether the data set constrains the model, avoid local minima of the objective function, and address the ill-posed nature of the problem if present.
Using this model we simulate excitation in a portion of the left ventricular free wall of a pig heart. The Bidomain Equations are solved with a Finite Element Method (resolution: 50 mm, 100 ms).
We show that 5 complementary stimulation protocols are necessary to fully constrain the sodium gating model. Data collection should be iterative, so stimulation parameters are adjusted to generate independent currents. When the data set is complete, the inversion recovers parameters and functions without ambiguity. However, the estimation is ill-posed with respect to sodium activation. A 5% white noise on the current generates up to 50% change on the time constant. When the data set is incomplete, the inversion identifies many models that can reproduce the data set. We come to realize that such models generate erroneous predictions. Varying the inactivation time constant within the range prescribed by the inversion, due to ill-posedness, predicts a variety vortex dynamics behaviors.