Multi-vesicular endosomes (MVEs) fuse with the plasma membrane to release exosomes into the extracellular space. The regulation and kinetics of this process is not well characterized, but probes for imaging MVE fusion have arisen recently. In particular, the design of an exosome marker with a pH sensitive dye in the middle of the tetraspanin protein CD63 has facilitated studies of individual MVE fusion events. Fusion events have been imaged using pHuji or pHluorin, while docking, protein accumulation during the fusion and docking process is imaged in another color channel using total internal reflection fluorescence microscopy. Fusion happens constitutively, in a relatively slow (~2 events per minute), and randomly located fashion. Fusion events exhibit a proportional relationship with temperature; decrease in temperature was accompanied by decrease in the rate of content loss and a decrease in the number of events per minute. Many of the fusion events release content in a biphasic decay with a component that lasts 10s of seconds, likely due to exosomal tethering on the cell surface, as others have observed in electron microscopy and fluorescence microscopy data. Through modeling of experimental data, insight into the tethering molecule can be deduced. The observed biphasic decay can be simulated with a fast, diffusive component where CD63 moves from the endosomal membrane into the plasma membrane and a second component of the decay arises from exosomes being secreted but tethered to the surface.

1117-Pos

Force redistribution during clathrin-mediated endocytosis is revealed by new phase-separating force sensors

Yuan Ren[1], Jie Yang[2], Huaizhou Jin[2], Yongli Zhang[2], Julien Berro[1].
[1]Department of Molecular Biophysics and Biochemistry, Yale University, West Haven, CT, USA, [2]Department of Cell Biology, Yale University, New Haven, CT, USA.

Forces exerted at the molecular level are central to countless processes in health and disease, yet measuring molecular forces *in vivo* remains difficult if not impossible using existing approaches. During clathrin-mediated endocytosis (CME), the plasma membrane is deformed into a vesicle by the forces produced by the actin cytoskeleton and transmitted to the membrane by a multi-protein coat. However, the actual forces required for endocytosis remain unknown. Here we present a new series of *in vivo* force sensors to measure the forces on the fission yeast HIP1R homologue End4p, a protein that links the endocytic membrane to the actin cytoskeleton. These new force sensors are based on calibrated coiled-coils that phase separate when they are under force. The measured forces on End4p are between 11 and 20 pN near the actin meshwork, between 10 and 11 pN near the clathrin lattice, and between 8 and 10 pN near the plasma membrane. Our results predict the participation of additional proteins to relay forces in different layers of the endocytic machinery during CME, and our approach points to a novel direction for *in vivo* force measurement.

1118-Pos

Rapid membrane flow at a presynaptic terminal

Carolina Gomis Perez[1], Natasha Dudzinski[2], Mason Rouches[2], Benjamin B. Machta[3], David Zenisek[1], Erdem Karatekin[4].
[1]Department of Cellular and Molecular Physiology, Yale University, West Haven, CT, USA, [2]Yale University, West Haven, CT, USA, [3]Department of Physics and Systems Biology Institute, Yale University, New Haven, CT, USA, [4]Nanobiology Institute, Yale University, West Haven, CT, USA.

Many cellular activities, such as cell migration, cell division, signaling, infection, phagocytosis and exo-endocytosis, generate membrane tension gradients that in turn regulate them. Moreover, membrane flows, which are driven by tension gradients, can limit exo-endocytosis coupling in space and time, as net membrane flow from exocytic to endocytic sites is required to maintain membrane homeostasis. However, there is controversy over how rapidly plasma membrane flows can relax tension gradients; contrary to the common view, recent work showed membrane tension does not equilibrate in several cell types. Here we show membrane tension can propagate rapidly or slowly, spanning orders of magnitude in speed, depending on cell type. In a neuronal terminal specialized for rapid synaptic vesicle turnover and where exo-endocytosis events occur at distinct loci, membrane tension equilibrates within seconds. By contrast, membrane tension does not propagate in neuroendocrine adrenal chromaffin cells secreting catecholamines. Thus, slow membrane flow and tension equilibration may confine exo- and exocytosis to the same loci. Stimulation of exocytosis causes a rapid, global decrease in the synaptic terminal membrane tension, which recovers slowly due to endocytosis. Our results demonstrate membrane tension propagates rapidly at neuronal terminals and varies during synaptic activity, likely contributing to exo-endocytosis coupling.

1119-Pos

Gaseous delivery of volatile anesthetics to a planar membrane

Ayden L. Olsen[1], Robert E. Coffman[2], Dixon J. Woodbury[1,2].
[1]Cell Biology and Physiology, Brigham Young University, Provo, UT, USA, [2]Neuroscience Center, Brigham Young University, Provo, UT, USA.

Volatile anesthetics are an important class of compounds that have become ubiquitous in healthcare. Current model systems used to explore the effects of these compounds in humans typically use tissue slices or cell culture. We are developing a simple protein-free assay to safely test the dose-dependent effects of volatile anesthetics on the fusion of liposomes to planar lipid bilayers. As an initial model, we use nitrogen gas bubbled through a concentrated ethanol solution to deliver ethanol vapor to an enclosed bilayer chamber, where the gaseous ethanol partitions into the chamber solution. Aqueous samples are taken over the span of the 40-minute experiment and measured by gas chromatography. Our data show that the flow rate of ethanol-saturated nitrogen gas and the stirring speed within the chamber heavily influence the rate of ethanol delivery. Initial experiments showed that the target concentration of 4% ethanol could be reached within 5 minutes of vapor delivery. As we have previously shown that ethanol alters fusion, delivery to the chamber will be further confirmed by measuring changes in membrane fusion. (Paxman, et al., 2017 Biophys J. 112:121-132). While our model currently has many limitations, our goal is to develop a fully contained assay that is safe to use with anesthetics such as diethyl ether and isoflurane, so as to further understand their effects on membrane fusion.

1120-Pos

Dynamics of Syntaxin at sites of constitutive and stimulated fusion

Alan W. Weisgerber, Melodie Nguyen, Anarkali Mahmood, Max Palay, Zdenek Otruba, Michelle K. Knowles.
Chemistry & Biochemistry, University of Denver, Denver, CO, USA.

Both stimulated and constitutive secretion events in cells, such as fusion of dense core vesicles and multi vesicular bodies, are facilitated by SNARE proteins. These biologically essential fusion events are driven by the clustering of SNARE complexes, however, the underlying molecular interactions that drive cluster formation during membrane fusion are not yet clear for different types of SNARE complexes. To characterize the accumulation of SNARE proteins into clusters, we visualized Syntaxin1a and Syntaxin4, two plasma membrane associated SNARE proteins, during docking and fusion events. The dynamics of cluster formation and protein mobility was measured at docked vesicle sites by tracking single molecules of Syntaxin in PC12 and A549 cells. These experiments were performed using two-color TIRF microscopy of nanobody and antibody labeled Syntaxin molecules. Single molecule dynamics were measured using anti-EGFP nanobody conjugated to AF594 for Syx1a-EGFP and Anti-myc antibody with AF594 for use with Syntaxin containing two extracellular myc tags. Fusion events were measured using EGFP or pHluorin labeled vesicles, while docking events were imaged using non-pH sensitive fluorescent proteins, such as mCherry. Through both temporally resolved, colocalization measurements of Syntaxin clusters at sites of fusion and docking and tracking of Syntaxin molecules, we address how clustering of SNARE molecules occurs for constitutive and stimulated fusion.

Posters: Cardiac, Smooth, and Skeletal Muscle Electrophysiology

1121-Pos

Human induce pluripotent stem cell-derived cardiomyocyte model to study PUFA analogs as a potential treatment of long QT syndrome

Alicia de la Cruz[1], Derek M. Dykxhoorn[2], Ashutosh Agarwal[3], Peter H. Larsson[1].
[1]Department of Physiology and Biophysics, University of Miami, Miami, FL, USA, [2]Department of Human Genetics, University of Miami, Miami, FL, USA, [3]Department of Biomedical Engineering, University of Miami, Miami, FL, USA.

Long QT syndrome (LQTS) is an inheritable ventricular arrhythmia that predisposes patients to torsades de pointes and sudden cardiac death. Mutations in voltage-gated ion channels and their regulatory β subunits involved in the cardiac action potential (AP) generate LQTS by prolonging the QT interval of the electrocardiogram. 15 types of LQTS (LQT1-15) have been described

depending on the underlying gene mutated. The majority of the cases correlate to LQT1-2 which are mutations in voltage-gated potassium channels involved in cardiac AP repolarization. LQT1 and LQT5 are described as loss-of-function mutations in genes that encode Kv7.1 (alpha pore-forming subunit) and its β-regulatory subunit KCNE1, respectively. Kv7.1/KCNE1 channels elicit one of the main repolarization currents involved in the cardiac AP. Therefore, Kv7.1/KCNE1 channel-specific activators might be a potential treatment for LQTS. Polyunsaturated fatty acid (PUFA) analogs can broadly modulate cardiac voltage-gated ion channels (Nav, Cav, Kv). Thus, PUFA analogs are described as potential anti-arrhythmic drugs. Linoleoyl-taurine (Lin-Tau) and docosahexaenoyl-taurine (DHA-Tau) are broadly selective and modulate multiple cardiac ion channels when applied on isolated channels in a heterologous system. In contrast, Lin-Glycine (Lin-Gly) and DHA-Glycine (DHA-Gly) showed higher selectivity for Kv7.1/KCNE1 channels compare to Nav or Cav. In the present study, we measured the effects of these four compounds, Lin-Tau, DHA-Tau, Lin-Gly, DHA-Gly, on human induce pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). hiPSC-CMs were grown on two different substrates, smooth and grooved substrates, to compare the effects of PUFA analogs on differently maturated hiPSC-CMs. Moreover, Kv7.1/KCNE1 channels blockers were applied to evaluate the maturation of hiPSC-CMs. We here use a combination of hiPSC-CM monolayers with a high-resolution, all-optical electrophysiology system for screening the anti-arrhythmic properties of diverse PUFA analogs.

1122-Pos

Sex-dependent differences in Ca^{2+}-related arrhythmia revealed by human atrial myocyte models

Xianwei Zhang[1], Charlotte Smith[2], Stefano Morotti[1], Eleonora Grandi[1], **Haibo Ni**[1].
[1]Pharmacology, University of California Davis, Davis, CA, USA, [2]University of Manchester, Manchester, United Kingdom.

Sex is an established independent risk factor for atrial fibrillation (AF), the most common cardiac arrhythmia. Substantial sex differences have been reported in prevalence, clini-cal representation, pathophysiology, therapy, and prognosis of AF; but the associated mechanisms and causes remain largely unknown. It is well established that AF is associated with remodeling of the Ca^{2+} handling system. A recent study in human atria revealed significant sex-differences in AF-related perturbations of Ca^{2+} handling, which could have important implications for understanding sex-specific AF pathophysiology and treatment. Here, we applied our recently developed computational model of human atrial electrophysiology and spatial Ca^{2+} signaling to systematically investigate sex differences in atrial arrhythmia propensity. We constructed both male and female models for sinus rhythm (SR) and AF conditions based on known AF-related and sex-dependent differences in the atrial Ca^{2+} handling. These models were subsequently integrated with our populations of subcellular ultrastructures describing various degrees of AF-induced remodeling in the transverse-axial tubular system (TATS). We performed simulations to determine the propensity of the male and female models to develop spontaneous Ca^{2+} release (SCR) events and delayed afterdepolarizations (DADs). Our results demonstrate an increased propensity for DADs and SCR events in AF vs SR, with the female vs male models exhibiting enhanced arrhythmia vulnerability due to increased ryanodine receptor (RyR) opening. The female-to-male difference in arrhythmia propensity is progressively enhanced with AF-induced remodeling of the TATS. Simulated restoration of the dysregulated RyRs in the AF populations substantially suppressed SCRs and DADs, especially in the female myocytes. Our study adds insight into sex-dependent differences in the Ca^{2+}-related arrhythmia propensity of the human atria and supports further exploration of sex-specific pharmacotherapies as a strategy for improving AF treatment.

1123-Pos

Electrophysiological characterization of mouse intracardiac calbindin neurons

Lizot Guénaëlle, Jocelyn Bescond, Patrick Bois, Jean-François Faivre, Aurélien Chatelier.
Signalisation et Transports Ioniques Membranaires Laboratory EA7349, Poitiers, France.

Neural control of the heart involves central and peripheral neurons that act interdependently to modulate cardiac parameters such as heart rate, conduction velocity or contractility. Within this cardiac neuronal regulation, the intrinsic cardiac nervous system, which correspond to clusters of neurons found on the dorsal atrial surface of the heart, is receiving growing attention. Indeed, whereas they were initially considered as simple parasympathetic postganglionic neurons, studies conducted over the past 30 years suggested a more complex organization, involving the existence of sensory, local regulatory and motor neurons within intracardiac ganglia. Moreover, growing evidence suggest the implication of this neural network in the initiation and maintenance of cardiac arrhythmias. However, the functional organization of this intracardiac neural network, as well as its involvement in cardiac diseases have not been fully elucidated.Therefore, this study aims to decipher the complexity of this mouse cardiac nervous system by examining the electrophysiological properties of intracardiac neurons. The characterization of passive and active electrical membrane properties of these neurons gave rise to the identification of two distinct neuronal profiles displaying different firing characteristics. The first group was classified as phasic due to its limited firing activity while the second was defined as adapting. Phasic neurons were also characterized by a higher rheobase as well as higher AHP amplitude and duration compared to the adapting one. By using cre transgenic mice and targeted viral transduction strategy, we identified calbindin expressing neurons as a population of neurons with a distinct electrophysiological signature. This could be explained by the differential expression of several ionic channels including sodium and calcium channels and will be further investigated in the future.

1124-Pos

Interpretable machine learning of action potential duration restitution properties in single-cell models of atrial cardiomyocyte

Euijun Song, **Young-Seon Lee**.
Applied Mathematics and Statistics, The State University of New York, Korea, Incheon, Republic of Korea.

Action potential duration (APD) restitution curve and its maximal slope (Smax) reflect single cell-level instability for inducing chaotic heart rhythms. Conventional parameter sensitivity analysis often fails to describe nonlinear relationships between ion channel parameters and electrophysiological phenotypes, such as Smax. We explored the parameter-phenotype mapping in *in silico* atrial cell models through interpretable machine learning (ML) approaches. We generated a population of 5,000 single-cell atrial cell models by log-uniformly sampling parameter combinations with the Sobol sequence scheme. Parameter sensitivity analyses could explain the linear relationships between parameters and electrophysiological phenotypes, including APD_{90}, resting membrane potential, Vmax, refractory period, and APD/calcium alternans threshold but not for Smax. But, neural network models had better prediction performance for Smax. To interpret the ML model, we evaluated the parameter importance at the global and local levels by computing the permutation feature importance and the local interpretable model-agnostic explanations (LIME) values, respectively. Increases in I_{CaL}, I_{NCX}, and I_{Kr}, and decreases in I_{K1}, $I_{b,Cl}$, I_{Kur}, I_{SERCA}, and I_{to} are correlated with higher Smax values. The LIME algorithm determined that I_{NaK} plays a significant role in determining Smax as well as I_{to} and I_{Kur}. The atrial population were hierarchically clustered into three distinct groups based on the LIME values. I_{NaK} had negative LIME values in clusters 1 and 3, but positive values in cluster 2. The single-cell simulation confirmed that the increase in I_{NaK} resulted in the higher Smax in cluster 2, but not in other clusters. Our combined top-down interpretable ML and bottom-up mechanistic simulation approaches uncovered the role of I_{NaK} in heterogeneous behaviors of Smax in the atrial population.

1125-Pos

Spatial and functional heterogeneity among pacemaker cell populations increases robustness and flexibility of SA node tissue pacemaker function in silico

Alexander V. Maltsev[1], Michael D. Stern[1], Edward G. Lakatta[2], Victor A. Maltsev[3].
[1]National Institute on Aging, NIH, Baltimore, MD, USA, [2]Laboratory of Cardiovascular Science, National Institute on Aging, NIH, Baltimore, MD, USA, [3]Intramural Research Program, National Institute on Aging, NIH, Baltimore, MD, USA.

Each heartbeat is initiated by pacemaker cells operating within the sinoatrial node (SAN). While SAN cells exhibit substantial spatial heterogeneity of their electrophysiological and Ca cycling parameters, the impact of this heterogeneity on SAN function has not been established. We investigated this problem numerically in a SAN tissue model computed by a GPU (25x25 cell grid), with each cell described by a coupled-clock Maltsev-Lakatta model. Action potential (AP) generation was examined in scenarios representing different cell populations with different degrees of spatial and