into how LTCs are coupled to cytoskeletal signaling pathways in neurons and shed light on the molecular mechanisms underlying the generation of TS and other Autism Spectrum Disorders.
Supported by NIH RO1 NS48564-01 to RD

1144-Plat
End-stage Mechanisms Underlying Voltage and Ca^{2+}/Calmodulin-Dependent Inactivation (VDI and CDI) of $Ca_V1.3$ Channels
Michael R. Tadross, Manu B. Johny, **David T. Yue**.
Johns Hopkins University, Baltimore, MD, USA.
The past decade has witnessed many discoveries about the early events that underlie calmodulin (CaM) regulation of Ca^{2+} channels. Much is known about the positioning of apoCaM (Ca^{2+}-free) on channels, and the initial Ca^{2+}/CaM interaction sites. Beyond this, precious little is clear about the eventual actions of this central genre of Ca^{2+} channel modulation. Does CDI involve hinged-lid occlusion, selectivity filter collapse, or allosteric inhibition of activation gating? Do CDI and VDI reach the same ultimate conformation? All these proposals remain in flux. Here, we deduce that mutations within the S6 activation gates would produce discriminating effects on activation and inactivation, depending on which mechanism holds true. For the first two end-stage mechanisms (hinged-lid occlusion and selectivity filter collapse), S6 mutations that enhance channel opening are predicted to strengthen channel inactivation. By contrast, for an allosteric mechanism, such mutations would actually weaken inactivation. These predictions motivated exhaustive mutagenesis of the S6 segments of all four homologous domains of $Ca_V1.3$. We find that S6 mutations affect VDI and CDI in strikingly different ways, indicating a fundamental divergence of their end-stage mechanisms. The pattern seen for CDI agrees remarkably well with that predicted for an allosteric mechanism. By contrast, VDI effects cannot be fully explained by any previously described end-stage mechanism. Instead, mapping the functional VDI effects onto a structural homology model of $Ca_V1.3$ reveals a telling structural pattern, suggestive of a novel 'hinged-lid-shield' mechanism. In this scheme, $Ca_V1.3$ channels feature a specialized distal S6 'shield' that repels lid closure. We validate this proposal with experiments in which the integrity of the shield and the mobility of the hinge-lid are independently modified. In all, these advances furnish a rich mechanistic backdrop for the many Ca^{2+} channelopathies involving S6 domains.

1145-Plat
Single Channel Conductance Of CaV2.2 At Physiological $[Ca^{2+}]_{Ext}$
Alexander M. Weber, Elise F. Stanley.
TWRI, UHN, Toronto, ON, Canada.
Ca^{2+} that enters through voltage-gated CaV2.2 channels and binds to a calcium sensor at the transmitter release site links membrane depolarization to activation of synaptic vesicle discharge. Recent evidence supports the hypothesis that the release site calcium sensor is within the single CaV2.2 channel domain. Thus, modeling presynaptic nanophysiology requires knowledge of the channel transport rate at physiological $[Ca^{2+}]_{ext}$. However, this value has only been determined previously for the non-presynaptic CaV1.x (L type) channel with a conductance of ~2.4 pS at $[Ca^{2+}]_{ext}$=2 mM (Church and Stanley, JP 1996). Since at $[Ba^{2+}]_{ext}$=100 mM CaV1.x has a conductance of ~24 pS while CaV2.2 has one of ~14 pS we predicted that at $[Ca^{2+}]_{ext}$=2 mM the latter channel would have a conductance of ~1.2 pS.
Single calcium channels were recorded using low noise, quartz electrodes from freshly isolated chick dorsal root ganglion neurons which express virtually entirely CaV2.2 current. In the presence of $[Ca^{2+}]_{ext}$=2 mM and 2 μM nifedipine, to block CaV1.x, and 0.1 mM Ni^{+} to block CaV3.X, together with standard Na^{+} and K^{+} channel blockers and n-methyl-D glucamine^{+} as the primary cation, we noted two single inward channel conductances: ~1.4 pS and ~2.5 pS (*N=4*). The larger channel was identified as CaV2.2 since it was absent in 4 of 4 patches with ω-conotoxin GIVA (2.5 μM), a specific CaV2.2 blocker, but was present in 7 out of 8 patches with 2 mM $[Ca^{2+}{}_{ext}]$ or $[Ba^{2+}{}_{ext}]$ whereas the small channel remained (*N=3*). Thus, our data indicate that at physiological $[Ca^{2+}]_{ext}$, CaV2.2 has a much higher conductance, and hence larger single channel domain, than predicted.

1146-Plat
Gating Charge Movement Is Prevented By Open State Occupancy Of N-type (CaV2.2) Calcium Channels
Viktor Yarotskyy, Keith S. Elmslie.
Penn State University College of Medicine, Hershey, PA, USA.
L-type calcium channels show a loose coupling between channel closing and gating charge movement. There are significant gating differences between N-type and L-type channels and we wondered if some of these differences were linked to the relationship between charge movement and channel opening. This was accomplished by comparing the time constant (τ) for channel closing (τ_{Deact}) with that for Off-gating charge movement (τ Q_{Off}) over a range of voltages. Ionic currents were recorded in 5 mM Ca^{2+}, while gating currents were recorded in 0.1 mM La^{3+} and 5 mM Mg^{2+} (La-Mg) from N-channels expressed in HEK 293 cells. τ Q_{Off} was larger than τ_{Deact} and the voltage dependence of the τ Q_{Off} was less steep than that for τ_{Deact}, which suggests that gating charge relaxation does not limit channel closing. To determine if the reverse was true, we used roscovitine, which slows N-channel closing by holding the channel in a high P_o open state. We found that τ Q_{Off} was identical to τ_{Deact} in roscovitine. There was a risk that residual ionic tail current could contaminate Off-gating current, so we used an envelope protocol to measured the recovery time course of Q_{On} (no ionic current contamination), and found the same τ as for both τ Q_{Off} and τ_{Deact} in roscovitine. This coincidence of τ Q_{Off} with τ_{Deact} suggests that transition out of the roscovitine-bound high Po open state becomes rate limiting to both Q_{Off} and channel closing. We conclude that, unlike L-channels, the high P_o N-channel open state places the channel into a confirmation that locks gating charge into the activated position.

1147-Plat
Cardiac $Alpha_{1a}$-adrenoceptor Stimulation Inhibits L-type Ca^{2+} Current In The Presence Of Beta-adrenoceptor Stimulation Through Tyrosine Kinase
Jin O-Uchi[1,2], Kimiaki Komukai[2], Satoshi Morimoto[2], Kenichi Hongo[2], Satoshi Kurihara[2].
[1]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [2]The Jikei University School of Medicine, Tokyo, Japan.
Introduction: We previously showed that cardiac α_1-adrenoceptor (AR) stimulation alone potentiates L-type Ca^{2+} current (I_{Ca}) through α_{1A}-AR-PLC-PKC pathway (O-Uchi J et al. ***PNAS.***, 2005 and ***Circ Res.***, 2008). However, the interaction of α_1- and β-AR signalings for I_{Ca} regulation was not fully clarified. In the present study, we examined the effect of α_1-AR stimulation on I_{Ca} when β-AR is stimulated. **Methods**: Perforated patch-clamp was used for recording I_{Ca} from isolated adult rat ventricular myocytes. Cells were at first treated with β-AR agonist (100 nM isoproterenol) for 5 min and then α_1-AR agonist (100 μM phenylephrine) was applied in the continuous presence of isoproterenol. Holding potential was set at -40 mV and depolarization pulse to 0 mV was applied every 10 sec. **Results**: Phenylephrine significantly inhibited I_{Ca} in the presence of isoproterenol by 19.6 ± 7.6%. The α_{1A}-AR selective antagonist (WB4101) blocked this inhibitory effect by phenylephrine, but α_{1B}-AR selective antagonist (L-765,314) did not, confirming that only α_{1A}-AR is involved in this inhibitory effect. Phenylephrine had no effect on I_{Ca} activated by forskolin. In addition, inhibition of G_q signaling by PLC inhibitor (U73122) or inhibition of $G_{i/o}$ signaling by pertussis toxin did not blocked the phenylephrine-induced inhibition of I_{Ca}. The tyrosine kinase inhibitor (lavendustin A) attenuated the response of phenylephrine during β-AR stimulation. **Conclusion**: α_{1A}-AR stimulation inhibits I_{Ca} in the presence of β-AR stimulation, which is opposite to the effect observed in the absence of β-AR stimulation. This effect is not mediated through G_q and $G_{i/o}$ but through tyrosine kinase activity, which inhibits the upstream of β-AR signaling (at the level of β-AR or Gs). The inhibitory effect of α_{1A}-AR stimulation could serve as one of the regulatory feedback mechanisms when catecholamine level increases under pathophysiological conditions.

1148-Plat
Calmodulin Regulates Calcium Sparklet Activity in Vascular Smooth Muscle
Manuel F. Navedo, Luis F. Santana.
University of Washington, Seattle, WA, USA.
Calcium influx through L-type calcium channels (LTCCs) influences numerous physiological processes in excitable cells ranging from contraction, memory and gene expression. Clusters of LTCCs can operate in a PKCalpha-dependent, high open probability mode that generates sites of sustained calcium influx called "persistent calcium sparklets". In vascular smooth muscle, persistent calcium sparklets contribute to local and global calcium. Calcium sparklets activity varies regionally within smooth muscle cells. At present however, the mechanisms underlying heterogeneous sparklet activity are incompletely understood. Here, we use TIRF microscopy and whole-cell patch clamp electrophysiology to investigate the role calmodulin in the modulation of calcium sparklet activity. We found that inhibition of calmodulin increases calcium sparklet activity in wild type (WT) smooth muscle cells. Inhibition of calmodulin in PKCalpha KO cells, which are devoid of persistent calcium sparklets, increased calcium influx by evoking new persistent calcium sparklet sites and by increasing the activity of previously low activity sites in these cells. On the basis of these finding, we hypothesize that calmodulin plays a critical role in determining the activity of calcium sparklet sites in arterial smooth