for the rapid upstroke of the action potential. The crystal structures of bacterial $Na_V$ channels that have been determined recently, have made way for studies of mammalian $Na_V$ channels through homology modeling. However, homology modeling is not straightforward because of the differences between mammalian and bacterial $Na_V$ channels. The Selectivity Filter (SF), which plays a key role in ion permeation, has EEEE residues in bacterial channel but has DEKA residues in mammalian channels. We have constructed a homology model for the $Na_V1.4$ channel from the crystal structure of $Na_VMs$ (PDB ID: 4CBC) bacterial channel. A recent study indicate that there are four stable inter-domain links between SF and neighboring domains. We additionally realized that there are extra residues making inter-domain links around the SF in $Na_VMs$ channel which are also conserved in the alignment between $Na_V1.4$ and $Na_VMs$ channel. The distances between the linked residues were used as restraints in our homology model. The time series of N-O distances have been obtained between linked residues from a 100 ns MD simulation which is performed using NAMD with the CHARMM36 force field. The N-O distances of extra links were found to be stable. We also studied channel stability, sodium ion hydration and coordination as well as the permeation mechanism. Comparison of the our simulation results with experiments show that our homology model provides a realistic representation of the mammalian $Na_V1.4$ structure.

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**Mechanism and Energetics of Ion and Tetrodotoxin Binding to NavMs Channel**

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Voltage-gated sodium channels (Nav) are important targets for treating various diseases. Crystal structure of the bacterial voltage-gated sodium channel NavMs in the open conformation has been obtained by Ulmschneider et al recently. We used this structure in our simulation work in order to study channel stability, sodium ion coordination as well as the ion permeation mechanism. We have employed free energy techniques to calculate the potential of mean force (PMF) for ion movement through the NavMs channel. The PMF calculations revealed the ion-binding sites in the channel and the mechanism of ion conductance. We also studied tetrodotoxin binding to the NavMs channel. Using docking and molecular dynamic simulations, we have constructed a model for the NavMs-tetrodotoxin complex. The toxin binds to various parts of the channel and occludes the ion-conducting pore. Our results help to explain experimental data and provide insights into the Nav inhibition process. The complex structures we have found provide templates for developing new sodium channel blockers with improved affinity and selectivity properties, which will be useful in the design of novel drugs targeting sodium ion channels.

## Voltage-gated Ca Channels I

**534-Pos Board B299**

**Modeling the Effects of Volatile Anesthetics on L-Type $Ca^{2+}$ Channels and $Ca^{2+}$ Induced $Ca^{2+}$ Release in Cardiomyocytes**

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Studies have shown that volatile anesthetics (VA; e.g. isoflurane) exert negative inotropic effects on cardiac cells, which could be one way in mediating cardioprotection against ischemia-reperfusion (IR) injury. Specifically, VA is shown to decrease the rate of $Ca^{2+}$ entry into the dyadic space via the L-type $Ca^{2+}$ channels (LCC), while also activating the ryanodine receptors (RyR) and $Ca^{2+}$ induced $Ca^{2+}$ release (CICR) process. However, the kinetic mechanisms of VA actions in these two processes and the consequent effects on the dyadic space $Ca^{2+}$ dynamics, which also regulate these two processes, are not well-understood. We developed here a computational model to investigate the effects of VA on $Ca^{2+}$ dynamics in the dyadic space, by extending and integrating contemporary kinetic models of the LCC and RyR, incorporating their regulations by VA. The extended kinetic model of the LCC systematically reproduces the various aspects of the LCC and its regulations by VA, observed experimentally; specifically, the VA inhibition of the LCC open probability and VA modulation of the LCC activation and inactivation gates that are mechanistically inferred through additional VA bound states of the LCC. Also, the extended kinetic model of the RyR is able to simulate the increased activity the RyR during VA exposure, as observed experimentally. The integrated computational model predicts the combined actions of the LCC and RyR and their modulations by VA on the dyadic space $Ca^{2+}$ dynamics and how the two factors collectively regulate the LCC and RyR kinetics during excitation-contraction (EC) coupling in cardiac cells during VA exposure. This mechanistic computational model provides a strong foundation for a whole-cell level model of cardiomyocytes $Ca^{2+}$ dynamics and electrophysiology during EC coupling and VA exposure during cardioprotection against IR injury.

**535-Pos Board B300**

**Electrophysiological Characterization of T-Type Calcium Channels in Central Medial Nucleus of the Rat Thalamus**

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Central medial nucleus (CeM) is a part of intralaminar thalamus that is involved in the control of arousal but mechanisms that regulate its activity are not well studied. It is well known that low-voltage-activated T-type calcium channels (T-channels) are abundantly expressed in the thalamus where they regulate neuronal excitability but their role in CeM was not previously investigated. Here, we investigated properties of T-channels in CeM using patch-clamp technique in acute coronal brain slices of adolescent rats. We used voltage-clamp recording with three different internal solutions: tetramethylammonium hydroxide (TMAOH) without ATP, cesium hydroxide (CsOH) with ATP and CsOH ATP-free solution. TMAOH internal solution induced a profound hyperpolarizing shift in steady-state inactivation curves of about 20 mV when compared to CsOH with ATP ($p<0.001$, one-way ANOVA). Smaller but significant shift of about 8 mV was achieved with CsOH ATP-free internal solution ($p<0.001$). In contrast, voltage-dependent steady-state activation kinetics of T-currents were not different under identical recording conditions. Additionally, we found that pan-selective T-channel blocker TTA-P2 at 5 μM decreased T-current density by about 70% ($p<0.001$, two-way RM ANOVA) and induced hyperpolarizing shift of 4 mV ($p<0.001$, t-test) in steady-state inactivation curves after 10 minutes of application. Moreover, we used current-clamp recordings to investigate the effects of TTA-P2 on firing patterns and passive membrane properties of CeM neurons. We found that TTA-P2 reduced tonic action potential frequency by 23% ($p<0.05$, two-way RM ANOVA), completely abolished rebound burst firing, reduced low-threshold spike (LTS) amplitude by 78% ($p<0.001$), slightly increased input resistance by 15% ($p<0.001$) and latency to LTS by 18% ($p<0.05$). Our results strongly suggest that T-channels are important regulators of neuronal excitability in CeM, which may be finely tuned by voltage-dependent phosphorylation. Supported by GM102525 to SMT.

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**Unmasking the Molecular Determinants Important for $Ca^{2+}$ -Dependent Regulation of $Ca_V2.2$**

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(CDI) and facilitation (CDF), respectively, which contribute to short-term synaptic plasticity. Both CDI and CDF are mediated by calmodulin (CaM) binding to sites in the C-terminal domain (CT) of the $Ca_v2.1$ $\alpha_1$ subunit, including a consensus CaM-binding IQ-domain. $Ca_v2.2$ (N-type) channels display CDI but not CDF but the underlying mechanism that blocks CDF in $Ca_v2.2$ is unknown. Here, we tested the hypothesis that $Ca_v2.2$ does not undergo CDF since it lacks essential molecular determinants for CDF that are present in $Ca_v2.1$. We find that alternative splicing of exons in the proximal and distal CT, which regulates CDF of $Ca_v2.1$, has no effect on CDF of $Ca_v2.2$. However, replacement of the entire CT of $Ca_v2.2$ with that of $Ca_v2.1$ produces robust CDF of the chimeric channel. Further analyses reveal that transfer of the $Ca_v2.1$ EF-hand, Pre-IQ- IQ domains, and a downstream CaM-binding domain (CBD) are sufficient to support CDF in chimeric $Ca_v2.2$ channels. Our results highlight the importance of the CT in distinguishing $Ca^{2+}$ feedback regulation of $Ca_v2.2$ and $Ca_v2.1$, and underscore how molecular distinctions may underlie the unique contributions of these channels in regulating neurotransmitter release.

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**C-Terminal Splice Variation Reveals New Insights into Calmodulin Regulation of $Ca_V1.4$ Channels**

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In synaptic terminals of retinal photoreceptors, $Ca_v1.4$ (L-type) $Ca^{2+}$ channels mediate $Ca^{2+}$ influx that promotes neurotransmitter release. Mutations