a pattern conserved in recombinant channels expressed in HEK293 cells. This clustering is dependent on the extent of channel phosphorylation. Our recent studies have shown that the clustered localization and gating properties of Kv2.1 are dynamically regulated by altered neuronal activity, ischemia, and by neuromodulatory stimuli via Ca2+/calcineurin-mediated dephosphorylation of the channel protein. These changes in Kv2.1 play a neuroprotective role. A number of studies have proposed the association of Kv2.1 with caveolar lipid rafts. We observed that Kv2.1 clusters in HEK293 cells do not overlap with caveolin1- or flotillin1/2-containing lipid rafts/microdomains on the cell surface. Moreover, caveolin1 staining/puncta were detected only in cultured rat astrocytes/glia, not in cultured rat hippocampal neurons. Caveolin1-RFP, overexpressed both in HEK293 cells stably expressing Kv2.1, and in cultured hippocampal neurons, exhibited distinct surface puncta that did not overlap with Kv2.1 clusters, and also did not alter the current density and voltage-dependent channel gating properties. Cyclodextrin-induced disruption of lipid rafts did not alter the clustered localization of Kv2.1 in HEK293 cells. Similarly, the staining pattern of Kv4.2 and Kv4.3 channels with or without the overexpression of KChIP2 did not overlap with caveolin1- and flotillin1/2-containing lipid rafts/microdomains in HEK293 and COS cells. These results suggest that in mammalian central neurons, somatodendritic Kv channels are not recruited to, and function independently of, lipid rafts/microdomains.

**2885-Plat**

**Stim-regulated Assembly And Stoichiometry Of The CRAC Channel Subunit Orai**

**Aubin Penna**, Angelo Demuro, Andriy V. Yeromin, Olga Safrina, Shenyuan L. Zhang, Ian Parker, Michael D. Cahalan.
UCI, Irvine, CA, USA.

Recent RNAi screens have identified Stim and Orai as critical components of the $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channel. Stim senses depletion of the Endoplasmic Reticulum (ER) $Ca^{2+}$ store, translocates from the ER to junctions adjacent to the plasma membrane (PM), and activates Orai pore-forming channel subunits in the PM to open the CRAC channel. The Orai oligomerization interface was investigated by co-immunoprecipitation of N-and/or C-terminal Orai deletion mutants and expressed Orai N- and C-terminal fragments. The transmembrane core domain plays a predominant role in subunit assembly; a weaker interaction interface was identified at the N-terminal region. We analyzed the quaternary structure of the Orai subunit and showed by cross-linking, and by non-denaturing gel electrophoresis that Orai is predominantly a dimer under resting conditions with or without co-expression of Stim. Single-molecule imaging of GFP-tagged Orai expressed in *Xenopus* oocytes revealed predominantly two-step photo-bleaching, consistent with a dimeric basal state. In contrast, co-expression of GFP-tagged Orai with the C-terminus of Stim as a cytosolic protein to activate the Orai channel without inducing $Ca^{2+}$ store depletion or clustering of Orai into punctae yielded predominantly four-step photobleaching, consistent with a tetrameric Orai stoichiometry of the active CRAC channel. Interaction of the Orai C-terminal coiled-coil domain (as shown by structure-disruptive mutations) with the C-terminus of Stim thus induces Orai dimers to dimerize, forming a tetramer that constitutes the $Ca^{2+}$-selective pore. This represents a novel mechanism in which assembly and activation of the functional ion channel are mediated by the same triggering molecule and may reveal a new channel gating mechanism. New data will be presented on the Stim-Orai stoichiometry and activation mechanism.

**2886-Plat**

**Protein Histidine Phosphatase 1 Negatively Regulates CD4 T Cells by Inhibiting the K+ Channel KCa3.1**

**Shekhar Srivastava**[1], Olga Zhdanova[1], Lie Di[1], Zhai Li[1], Mamdouh Albaqumi[1], Heike Wulff[2], Edward Y. Skolnik[1].
[1]NYU School of Medicine, New York, NY, USA, [2]University of California, Davis, CA, USA.

The calcium activated K+ channel KCa3.1 plays an important role in T lymphocyte Ca2+ signaling by helping to maintain a negative membrane potential which provides an electrochemical gradient to drive Ca2+ influx. We previously showed that Nucleoside Diphosphate Kinase Beta (NDPK-B), a mammalian histidine kinase, is required for KCa3.1 channel activation in human CD4 T lymphocytes. We now show that the mammalian protein histidine phosphatase (PHP)-1 directly binds and inhibits KCa3.1 by dephosphorylating histidine 358 on KCa3.1. Overexpression of wild type, but not a phosphatase dead PHPT-1 inhibited KCa3.1 channel activity. Decreased expression of PHPT-1 by siRNA in human CD4 T cells resulted in an increase in KCa3.1 channel activity and increased Ca2+ influx and proliferation following T cell receptor (TCR) activation indicating that endogenous PHPT-1 functions to negatively regulate CD4 T cells. Our findings provide the first example of a mammalian histidine phosphatase negatively regulating TCR signaling and are one of the few examples of histidine phosphorylation/dephosphorylation influencing a biological process in mammals.

**2887-Plat**

**Targeting The Voltage Sensor of Kv7 channels: Novel Strategies to Cure Hyperexcitability Disorders**

Asher Peretz[1], Liat Pell[1], Yana Gofman[2], Yoni Haitin[1], Nir Ben-Tal[3], **Bernard Attali**[1].
[1]Sackler Medical School, Tel Aviv University, TelAviv, Israel, [2]Faculty of Life Sciences Tel Aviv University, TelAviv, Israel, [3]Faculty of Life Sciences, Tel Aviv University, TelAviv, Israel.

The pore and gate regions of ion channels have been often targeted with drugs acting as channel blockers or openers. In contrast, the voltage sensing domain (VSD) was practically not exploited for therapeutic purposes. We recently designed a series of novel diphenylamine carboxylate derivatives to generate powerful Kv7.2 channel openers and blockers. Openers like the compound NH29 with aromatic nitro groups, robustly increased Kv7.2 $K^+$ currents. In sensory DRG and hippocampal neurons, the opener depressed evoked spike discharges. NH29 dampened hippocampal glutamate and GABA release thereby inhibiting spontaneous EPSCs and IPSCs. *In vivo*, these openers exhibited anti-convulsant activity. To identify the target residues involved in the action of NH29 we designed various mutations of Kv7.2 channels and checked the potency of NH29 using the whole-cell patch-clamp technique in CHO cells. We found that the mutants S121A at S1-S2 loop and L197G, R198A, R201A, R207W and R214W at S4 helix are significantly less sensitive to the activating effect of NH29 compared to WT Kv7.2 channels. Interestingly, our results indicate that NH29 does not act at the same target site as the opener retigabine (W236) and zinc-pyrithione. Docking experiments suggest that the nitro functionality of NH29 acts as a H-bonding acceptor which interacts with the guanidinium group of arginine 207 of the S4 helix. Thus, the new Kv7.2 channel opener NH29 acts as a gating modifier which interacts with the externally accessible surface of the VSD. NH29 docks to the groove formed by the interface between S4 helix and S1-S2, thereby stabilizing the VSD in the activated conformation. Our research is expected to generate a new generation of gating-modifiers specifically targeted to the VSD of potassium channels for the treatment of hyperexcitability disorders.

**2888-Plat**

**Regulation of the NALCN Sodium Leak Channel by Neuropeptides**

Boxun Lu, Yanhua Su, Sudipto Das, Haikun Wang, Yan Wang, Jin Liu, **Dejian Ren**.
Univ Pennsylvania, Philadelphia, PA, USA.

Several neurotransmitters act through G-protein coupled receptors (GPCR) to evoke a ''slow'' excitation of neurons. These include peptides, such as substance P (SP) and neurotensin (NT), as well as acetylcholine and noradrenaline. Unlike the fast (~ ms) ionotropic actions of small molecule neurotransmitters, the slow excitation is poorly understood at the molecular level, but can be mainly attributed to suppressing $K^+$ currents and/or activating a non-selective cation channel. Several $K^+$ channels, including members in the $K_V7$ subfamily, are inhibited by the neurotransmitters in a G protein-dependent fashion. The molecular identity of the cation channel has yet to be determined; similarly how the channel is activated and its relative contribution to neuronal excitability induced by the neuropeptides are unknown. We show that, in the hippocampal and ventral tegmental area neurons, SP and NT activate the voltage-independent $Na^+$-leak channel NALCN, a unique member in the 4×6TM channel family that also includes the voltage-gated $Ca^{2+}$ ($Ca_V$) and $Na^+$ ($Na_V$) channels. The activation by SP through NK1R (a GPCR receptor for SP) is not blocked by the non-hydrolyzable GTP or GDP analogs. These findings identify NALCN as the cation channel activated by SP receptor, and suggest that a G protein-independent mechanism is involved in the coupling from receptor to channel.

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**Role of Aromatic Residues for Local Anaesthetic Binding to Ion Channels**

**Nilsson Johanna**, Henrik Ullman, Kristoffer Sahlholm, Peter Arhem.
Dept of Neurosci, Stockholm, Sweden.

Local anesthetic and antiepileptic drugs acting on Nav and hERG channels have been assumed to bind to aromatic residues in the internal vestibule; to 1764F and 1771Y in Nav (Liu et al., 1996) and to 652Y and 656F in hERG (Mitcheson et al., 2000). Despite a lack of such residues in Kv channels, some local anaesthetics (e.g. bupivacaine) bind to Kv channels with a considerable affinity. To explore the role of aromatic residues for local anaesthetic binding we investigated the effect of bupivacaine on mutated Shaker channels (P466F and V473Y; I470Y and P474F), expressed in Xenopus oocytes, with a voltage clamp technique.