along with the corresponding charge valence. The phosphorylation states are specified through charges assigned to the serine amino acids of the Lys-Ser-Pro (KSP) repeat motifs of the side-arms. The equilibrium structure of the neurofilament brush has been studied via the model that maintained the proper charge distributions and grafting density of neurofilament side arms. It has been found that in spite of extensive phosphorylation sites present on NF-H, the tails of the medium sized neurofilament subunit (NF-M) is more elongated than NF-H tails. This suggests that NF-M protrusions are more critical in regulating neurofilament spacings and axonal caliber.

2475-Pos Board B445
Efficient Coding in the Olfactory Receptor Neuron Signaling Pathway
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The efficiency of neuronal coding has been studied extensively within the context of spike trains. Significantly less attention has been paid towards coding efficiency in biological signaling pathways. This study applies Shannon information theory to the olfactory receptor neuron signaling pathway to determine under what conditions the olfactory system can code most efficiently. We explore which types of odor stimuli the vertebrate olfactory system is most proficient at encoding by analyzing simulated data from a computational model of the pathway. We focus on odor stimuli of constant length but of varying concentration. This study concludes that the olfactory system's ability to encode such stimuli decreases significantly when presented with odor pulses of length greater than one second. We further explore the roles of particular signaling molecules in contributing to this decrease in coding efficiently. Finally, we perform a parameter sensitivity analysis on our information-theoretical calculations to identify the mechanisms responsible for information bottlenecks. We found that variations in upstream mechanism rate coefficients such as the G-protein activation rate have a significant effect on the transmission of information over stimuli longer than one second. In addition, parameter variations of the calcium extrusion rate through the sodium-calcium exchanger had a significant effect on information transfer over all pulse lengths.

2476-Pos Board B446
Reptitive Firing In Neurons - Analysing The Interaction Between Channel Density And Kinetics In Membrane Models
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More than sixty years after Alan Hodgkin presented his classification of firing patterns in the axons of the crab Carcinus maenas, the underlying mechanisms of the firing patterns are still only fragmentarily understood. Two main types have been discerned in neurons and dynamical membranes models. Type 1 shows a continuous frequency-stimulation current (f-I) relationship and thus an arbitrarily low frequency at threshold current, while Type 2 shows a discontinuous f-I relationship and a minimum frequency. Type 1 obtains rhythmicity via a saddle-node bifurcation, thus requiring three stationary potentials at subthreshold stimulation current. Type 2 obtains rhythmicity via a Hopf or double-orbit bifurcation. In a previous investigation of a hippocampal neuron model we showed that the membrane density of critical ion channels could regulate the bifurcation type and consequently the threshold dynamics. In the present study we extend our previous analysis to other quantitatively well-described excitable membranes. These studies show that not merely the channel density, but the overall structure of the phase space around the stationary potentials determine the onset frequency. We show, by means of techniques from nonlinear dynamical system theory, that this phase space is altered both by changes in channel density and channel kinetics. Understanding these interactions is an important step towards understanding global oscillatory activity in brain networks.

2477-Pos Board B447
How Can BK Channels Increase Excitability of Central Neurons and Decrease Excitability of Nodose Neurons?
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K^+ currents are generally known to hyperpolarize cells and to inhibit neuronal excitability. Thus, in nodose neurons, BK channel inhibition *increases* excitability (Snitsarev et al. J.Physiol. 582, 177). In central neurons, however, *increased* BK activity leads to *increased* excitability (Brenner et al. Nat.Neurosci. 8, 1752). To gain an insight into this opposing physiological effect of BK channel, we used Simulink (Mathworks) to perform mathematical modeling of action potential (AP) generation:
http://www.mathworks.com/matlabcentral/fileexchange/loadFile.do?objectId=18812&objectType=file.
In response to simulated depolarization with 0.04 nA current injection, the simulation generated 4 APs adapting within 200 ms. Doubling BK conductance from 0.0065 to 0.013 microS resulted in 12 APs adapting within 600 ms, and halving BK conductance to 0.00325 microS resulted in 3 APs adapting in less than 200 ms. Thus, contrary to our expectations, an increase in excitability resulting from increased BK current was obtained in the nodose neuron model system. This behavior is reminiscent of central neurons. Other K^+ conductances and their effects on excitability in the nodose neuron model were also tested. In line with current experimental and theoretical knowledge, an increase in A-, K-, or D-current resulted in expected decrease in excitability (Schild et al. J. Neurophysiol. 71, 2338). To reconcile the experimental data from nodose neurons, central neurons and mathematical models of these neurons, we are introducing into our models recently discovered endogenous inhibition of BK current by a toxin-like domain of acid-sensing ion channels (Petroff et al. PNAS 105, 3140) and its competition with experimentally added scorpion toxins.
Mathematical models of neuronal excitability, and especially involvement of K^+ channels, may help our understanding of altered excitability of central neurons in epilepsy, neurodegenerative and psychiatric diseases, and decreased excitability of baroreceptor nodose neurons in hypertension and heart failure.

2478-Pos Board B448
New Metrics of Intrinsic Axonal Excitability from a Computational Model of Demyelination
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In white matter, oligodendrocytes tightly wrap axons at regular intervals to form the myelin sheath, the primary attribute of which is conduction velocity acceleration. Axonal demyelination diseases represent a devastating group of neurological disorders that affect more than 2 million people annually worldwide. The process of unraveling the periodic insulation causes axon conduction dysfunction in many diseases of the central nervous system (CNS), as in multiple sclerosis (MS) and infectious encephalomyelitis, or the peripheral nervous system (PNS) as in Guillain-Barré or Charcot-Marie-Tooth syndromes. Although the etiology of these diseases in most cases is thought to be immunological, the mechanisms of the diverse neurological symptoms are just as poorly understood. These confounding symptoms can present intermittently, resolving and returning in a way that is desynchronized from re-myelination. Symptoms include spasticity, dysfunction of somatic sensation, motor control, impairment of vision and other modalities. But these multiple neuropathies cannot be understood by conduction velocity changes alone. Physiological features are accompanied by anatomical and cellular perturbations in affected neurons that include changes in voltage-gated ion channel densities.
Here we present a compartmental model of a partially demyelinated axon using the NEURON simulator (http://www.neuron.yale.edu/neuron/) that sheds light on the function of normal, healthy axons as well as those undergoing demyelination. The model suggests a simple set of rules that could explain the wide range of intermittent symptoms observed during demyelination. The rules that govern these destabilized excitability patterns are critically dependent on ion channel densities and the anatomical parameters of the axon. Support: HHMI, NIH R01-MH079076.

2479-Pos Board B449
Light-dark Cycle Memory In The Mammalian Circadian Clock
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The mammalian circadian oscillator, or superchiasmatic nucleus (SCN) contains several thousand clock neurons in its ventrolateral (VL) part, many of which are spontaneous oscillators with periods that range from 22 to 28 hours. In complete darkness this network synchronizes through the exchange of action potentials which release the neuropeptide VIP, striking a compromise, free-running period (FRP) that is close to 24 hours long. We lock Siberian hamsters to various light-dark cycles and then track their activity into the dark to show that they retain a memory of the particular cycle to which they were entrained before returning to their own FRP. Then using model clock neurons (1) we model the VL SCN network and show that strong rythmicity of the VIP oscillation can account for both synchronization in darkness and the light-dark cycle memory which we observe. Additionally, light is known to initiate a MAP kinase cascade that induces transcription of both per and mkp1 phosphatase. We show that the phosphatase-kinase interaction can account for the dead zone in the mammalian Phase Response Curve. Finally, we hypothesize that the SCN acts like a lock-in amplifier to reject noise and to entrain the light edges of the circadian day.

(1) Leloup, J-C. and A. Goldbeter. 2003. Toward a detailed computational model for the mammalian circadian clock. PNAS 100:7051-7056.

Voltage-gated K Channels - Gating II

2480-Pos Board B450

Gate Opening Remotely Controls the Interaction between the Voltage Sensor and the Cytosolic Domain in BK Channels

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In voltage- and ligand-gated ion channels the voltage sensor domain (VSD) and the ligand sensor change conformation upon stimulation, which then triggers the activation gate to open. However, the retrograde control of the conformation of the sensors by the activation gate has not been well studied. Recently, we reported that Mg2+ binds to the interface between the cytosolic domain and the membrane-spanning VSD of BK type Ca2+-activated K+ channels and activates the voltage sensor through an electrostatic interaction (Yang et al., 2007; Yang et al., 2008). Here we show that the interaction between Mg2+ and the voltage sensor is controlled by the opening of the activation gate. A mutation (F315A) in the middle of the pore-lining S6 segment altered channel opening such that the channels did not open even though the voltage sensor was fully active. The lock of the channel at the closed conformation also abolished the electrostatic interaction between Mg2+ and the VSD. 100 μm [Ca2+]i opened the activation gate of the mutant channel and reestablished the electrostatic interaction. Therefore, the activation gate and the sensory domains in BK channels are allosterically coupled and undergo concerted movements during channel gating.

References:
Yang, H. et al. 2007. Proc. Natl. Acad. Sci. U. S. A. 104:18270-18275.
Yang, H. et al. 2008. Nat. Struct. Mol. Biol. (In press).

2481-Pos Board B451

Cooperativity Between Voltage-sensing Domains in the Human BK Channel Revealed by Voltage-clamp Fluorometry.

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Like other members of the voltage-gated K+ channel superfamily, BK channels are thought to derive voltage sensitivity from charge-possessing transmembrane segments S2-S4. Particularly in BK, S2 is thought to have a direct role in voltage sensing (Ma et al., JGP2006). We combined cut-open oocyte voltage-clamp with fluorometry, after labeling unique cysteines introduced in cysteine-less BK channels (hSlo) with TMRM (Savalli et al., PNAS2006 and JGP2007), to resolve voltage-dependent conformational rearrangements near the extracellular side of S2. The intensity of fluorescence emission (ΔF) was strongly voltage-dependent (FV_{half}=-92 ± 2.7mV, Fz=0.95 ± 0.07, n=8), reporting protein rearrangements. To investigate voltage sensor function, we targeted two putative voltage-sensing residues: D153 (S2) and R213 (the single voltage-sensing residue in S4 -Ma et al., JGP2006). Neutralizing D153 in S2-labeled channels abolished voltage-evoked fluorescence deflections, strongly supporting the role of D153 in voltage activation of S2. Neutralizing R213 in the S4-labeled channel gave rise to a detectable but weakly voltage-dependent ΔF (Fz<0.2, n=2), perhaps arising from the S3 charge (D186, Ma et al., 2006). Cooperativity amongst voltage sensing transmembrane segments was evaluated by investigating ionic currents and ΔF from an S4 charge mutant labeled in the S2 and *vice versa*. In both cases, protein rearrangements were detected, albeit less voltage-dependent (Fz=0.21-0.23, n=5-6 respectively). These findings revealed that strong S2-S4 cooperativity underlies voltage sensing in the intact channel. In contrast to fluorescence experiments, the change in voltage sensitivity of ionic currents was smaller (Gz=0.70-0.86), supporting the view that the pore has intrinsic voltage dependence. An allosteric model of gating composed of two types of voltage sensing tetramers surrounding a single pore was used to provide a global fit of the experimental results.

2482-Pos Board B452

Structural and Functional Analysis of the Purified Cytosolic C-Terminus of the Human BK Channel

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The large-conductance voltage- and Ca^{2+}-activated K^+ channels (BK) are activated by membrane depolarizations and intracellular Ca^{2+}. Two consecutive intracellular RCK (Regulators of K^+ Conductance) domains, RCK1 and RCK2, are responsible for calcium sensitivity and together form the majority of the C-terminus.

Information about the structural and functional properties of the individual RCK1 and RCK2 domains has been reported by our group (Yusifov, et. al. PNAS 2008). However, the characterization of the full C-terminus remains unresolved. Here we report the initial structural and functional characterization of a high-purity protein expressed and purified from *E. coli* corresponding to the human (hSlo) BK channel C-terminus. The expressed C-terminus includes 684aa, starting from the S6-RCK1 linker and encompassing RCK1, RCK2, and the interconnecting 92aa RCK1-RCK2 linker.

The calcium-binding activity of the C-terminus (10ug), loaded on a nitrocellulose membrane, was probed by dot blot analysis of $^{45}Ca^{2+}$-binding. The C-terminus displayed a strong calcium-binding property when compared to Albumin.

The organization of the secondary structure of the C-terminus was investigated using Circular Dichroism (CD) spectroscopy. Far-UV CD spectra (190-260nm) of the C-terminus, analyzed with CONTIN/LL algorithm from the CDpro suite (SMP56 protein reference set), gave a secondary structure consisting of 29% α-helix, 20% β-strand, 22% turn, and 29% unordered.

The quaternary structure of C-terminus was investigated using size-exclusion chromatography with a Superdex 200 10/300 column. The C-terminus eluted in a single peak at a molecular weight of 330kDa corresponding to the theoretical tetrameric C-terminus complex (310kDa). In denaturating condition (SDS-gel electrophoresis), the C-terminus migrated as a monomeric 74kDa band (expected 77.6kDa).

In conclusion, we have successfully purified the functional human BK channel C-terminus domain, which allows for further investigation of the properties of the mammalian BK ''Gating ring,'' encompassing eight RCK domains.

2483-Pos Board B453

Calcium Sensing Properties of the RCK1 Domain of the Human BK Channel: Effects of the D362/367A Mutation

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Recent studies suggest that Ca^{2+}-dependent activation of the large-conductance voltage- and Ca^{2+}-activated K^+ (BK) channel is controlled by two RCK (Regulators of K^+ Conductance) domains located in the C-terminus, encompassing high affinity Ca^{2+}-sites D362/367 and M513 in RCK1, and a Ca^{2+}-bowl (D894-898) in RCK2 (Schreiber, et al, 1999, Lingle et al. 2002, Bao et al. 2004). Previously, we characterized Ca^{2+}-induced conformational changes and the role of the Ca^{2+} bowl in the hSloRCK2 domain using solution-based analysis (Yusifov et al., PNAS 2008).

Using a similar approach, we are now investigating the Ca^{2+}-dependent properties of the WT and D362/367A mutations of a purified protein corresponding to the amino acid sequence (322*IIE¼ DPL*667) that forms the human BK channel's RCK1 domain.

The calcium-binding activity of purified RCK1 (10ug), loaded on a nitrocellulose membrane, was directly probed by dot blot analysis of $^{45}Ca^{2+}$-binding. Albumin and Troponin were used as negative and positive controls, respectively. RCK1 showed remarkable calcium-binding ability when compared to Albumin.

Circular Dichroism (CD) analysis of the WT-RCK1 revealed a calcium-dependent spectral change, corresponding to an increased β-strand content of ~9% as the free [Ca^{2+}] was increased from 0.015 to 31.2μM. This change was paralleled by a similar decrease in α-helix content, while the turns and unordered fractions remained practically unchanged.

On the other hand, the CD spectra of RCK1-D362/367A mutant displays ~7-8% increased beta content, similar to the calcium-bound form of WT-RCK1. The Far-UV CD spectra obtained of hSloRCK1-D362/367A mutant in increasing free Ca^{2+} displayed no changes, suggesting a lack of substantial Ca^{2+}-dependent structural changes. Based on these findings, we propose that the D362/367A mutation in hSloRCK1 may lead to a conformational state of hSloRCK1 that is unable to translate Ca^{2+}-binding to channel gating.

2484-Pos Board B454

Cholesterol-ethanol Interactions On Vascular Myocyte BK Channels: Contribution To Alcohol-induced Cerebrovascular Constriction

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The activity of large conductance, calcium- and voltage-gated potassium (BK) channels critically limits the degree of vascular smooth muscle contraction, favoring cerebrovascular dilation (Brayden and Nelson, 1992). Ethanol at levels reached in circulation after moderate binge drinking (50 mM) inhibits the activity of cerebrovascular myocyte BK channels, leading to endothelium-