unwarranted) assumption that only persistent and not transient I_{Na} contributes to Na^+-loading and thence to discharge energetics. Even at their highest discharge frequencies, electrocytes produce full amplitude APs driven by transient I_{Na}. To make the accounting of electrocyte ion homeostasis robust, we describe contributions from the V-dependent Na-conductance with as much precision as is practicable.

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The Mechanism of Inhibition of Necrosis by Humanin Derivatives: A Potential Treatment for Ischemia and Related Diseases

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In the past, necrosis, which is associated with many diseases was considered to be an uncontrolled process; however, recent data support the notion that necrosis is a regulated process, yet, the only available treatment for necrosis is applying high oxygen pressure. Humanin (HN) is a 24-amino acid peptide, known for its anti-apoptotic activity, especially against neuronal cell death by Alzheimer insults. Recent studies showed that HN has other protective actions such as in myocardial ischemia, atherosclerosis and more; most of the activities were related to anti-apoptotic action. HN was also shown to increase cellular ATP levels. Our study reveals that, in addition to their anti-apoptotic activities, HN and some of its peptide derivatives exhibits a protective effect against necrotic insults in neuronal cell lines (PC-12 and NSC-34). Additionally, we found that HN affects ATP levels in cells undergoing necrosis, and interacts directly with mitochondrial ATP synthase enhancing its activity. Results obtained by fluorescence lifetime imaging microscopy (FLIM) and super-resolution microscopy with fluorescein-labeled HN, support the aforementioned findings. Furthermore, in vivo studies in traumatic brain injury on C57BL/6J mice, show a protective effect of HN, as demonstrated by improved motor performance and by MRI study done in a 1 Tesla small animals device. Thus, the present study may reveal a novel anti-necrotic mechanism of action of HN and its derivatives, and provide a new strategy for potential therapeutic treatment of ischemic states.

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Coarse-Grained Model of the Snare Complex Determines the Number of Snares Required for Docking

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Synaptic transmission requires that vesicles filled with neurotransmitter molecules be docked to the plasma membrane by the SNARE protein complex. The SNARE complex applies attractive forces to overcome the long-range repulsion between the vesicle and membrane. To understand how the balance between the attractive and repulsive forces defines the equilibrium docked state we have developed a model that combines the mechanics of vesicle/membrane deformation with a new coarse-grained model of the SNARE complex. The coarse-grained model of the SNARE complex is calibrated by comparison with all-atom molecular dynamics simulations as well as by force measurements in laser tweezer experiments. The model for vesicle/membrane interactions includes the forces produced by membrane deformation and electrostatic repulsion. Combining these two parts, the coarse-grained model of the SNARE complex with membrane mechanics and electrostatics, we evaluate the number of the SNARE complexes needed to maintain the equilibrium docked state. We find that a single SNARE complex is able to bring a typical synaptic vesicle to within a distance of about 2.5-3.5 nm from the membrane, which may be sufficient to trigger fusion. Further addition of SNARE complexes shortens this distance to 1.5-2.0 nm, but addition of more than six SNAREs actually increases it. Thus, our results indicate that 3-6 SNARE complexes work most efficiently to stabilize the docking process, and that synaptic vesicles and the membrane in a docked pre-fusion state are separated by approximately 1.5-2 nm.

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Organization and Dynamics of Nicotinic Acetylcholine Receptor Nanoclusters at the Cell Surface

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Organization and maintenance of neurotransmitter receptor assemblies, key for efficient synaptic transmission, rely on an adequate balance of synthesis, delivery to and removal from the cell membrane. We are studying the supramolecular organization, dynamics and trafficking of neuronal and muscle-type nicotinic acetylcholine receptors (nAChRs) using a combination of ensemble averaging methods and single-molecule experimental techniques. STED and GSDIM microscopies (two forms of nanoscopy) of fixed specimens of a clonal cell line expressing muscle-type AChR disclosed diffraction barrier-breaking light microscopy images of ~55 nm cell-surface particles (''nanoclusters'') which increase in size upon ligation. Live cell imaging has enabled the visualization of particles in real time, and single-particle tracking together with cluster analysis reveals the motional regimes and modes or supramolecular assembly of nAChR nanoclusters. The neutral lipid cholesterol and cortical actin dynamics act synergically to modulate the supramolecular organization and dynamics of nAChRs at the cell surface.

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Molecular Modeling and Interaction Analysis of Cannabinoid Receptor Interacting Protein CRIP1b

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The Human Cannabinoid receptor interacting protein (CRIP1b), a novel protein, is encoded by Cnrip gene loacted on chromosome number 2. CRIP1b is involved in modulation of CB1 cannabinoid receptor's cellular localization and signal transduction but its structure and function remains uncharacterized. The present study has been aimed at investigating three dimensional structure in an effort to provide clues to CRIP1b function. An ab initio modeling approach for prediction of CRIP1a structure has been applied since CRIP1b protein does not share any significant sequence homology to other proteins. The best model obtained from modelling studies for CRIP1b has a compact structure of beta sheets along with a small helix and loops, which is supported by zDOPE score, secondary structure data, Ramachandram plot, and other validation methods. Since CRIP1b protein interacts with the last nine amino acids of the C-terminus of the human CB1 Cannabinoid receptor, the interactions between CRIP1b and a CB1 distal C-terminus nine-mer peptide were studied by performing molecular docking. The best model obtained was docked with CB1 C-terminal peptide, followed by molecular dynamics simulations to investigate binding and relative stability between CB1 and CRIP1a. Molecular docking revealed the involvements of a few hydrogen bonds in binding pocket of CRIP1b. Further the entire complex was optimized by 20 ns MD simulation with Gromacs4.5.5 and the rearrangements of hydrogen bonds leading to structural changes. The relationship between Cannabinoid receptor isoform one (CB1) and CRIP1b have potential to play significant roles in pain management, addiction, cardiovascular diseases, diabetes, and neurodegenerative disorders.

770-Pos Board B550

Physical and Topological Constraints on Growth in Human Brain Networks

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Noninvasive MRI technology enables the mapping of white matter connectivity between cortical regions in the human brain. Graph theoretic analysis has shown the organization of this structural brain network facilitates certain functional properties, such as robust and efficient transfer of information between brain regions. What are the generative mechanisms of such a network? To address this, we analyze a broad range of synthetic networks produced by simple growth rules, and we compare these networks both qualitatively and quantitatively to the brain. We consider a growth model in which a set of three parameters govern the tendency of nodes to form connections with one another. These generated networks are analyzed in terms of direct measures of connectivity, such as the length and number of network connections, as well as higher-order graph metrics, such as hierarchy and clustering, that relate to efficient information transfer and resistance to random errors. We find that different sets of input parameters can greatly affect the final structure of the network, with only some subsets of parameter values creating brain-like networks. This shows that very similar growth rules can produce a diverse set of network structures. In ongoing work, we are investigating the role of known biophysical constraints, such as

bilateral symmetry and wiring cost, on network growth. This study lends insight into the biophysical mechanisms that govern the development of human brain networks and ultimately shape robust and efficient network function.

771-Pos Board B551
Unified Model of Synaptic Transmission
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Learning and memory are two of the most fundamental and widely studied processes which underlie neuroscience. The mechanisms facilitating these processes are collectively known as synaptic plasticity. Through the modulation of synapse strength depending on the activity of neurons, synaptic plasticity reshapes the neural network over time. Synaptic plasticity is caused by the release of glutamate from pre-synaptic neurons and the activity of the synapse is mediated by glutamate receptors (AMPA and NMDA), excitatory amino acid transporters (EAAT), Calmodulin (CAM) and related protein kinases (CAMKII) and phosphatases (PP1). The crucial mechanism is that the ion transport into the post-synaptic compartment through ionotropic glutamate receptors (iGluRs) modulates the level of Na^{+} and Ca^{++} conductance as well as the number of iGluRs that migrate to the postsynaptic cell membrane. In this study, a unified model incorporating pre-synaptic and post-synaptic transport is developed. The limitations on the simulations due to the complexity and the size of the model are eliminated by coarse-graining in the model and by using hybrid particle/population simulation tools. The effects of different initial concentrations of Na^{+} and Ca^{++} and neurotransmitters (glutamate) in different compartments are examined in order to understand how this variables, which may be altered in neurodegenerative diseases such as Alzheimer's and Huntington's, affect synaptic transmission. Overall, the model and simulations are expected to provide insight into new therapeutic strategies.

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Structures of Human Miro1 Reveal Conformational Changes
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The outer mitochondrial membrane protein Miro is a highly conserved calcium-binding GTPase that is at the regulatory nexus of several processes, including mitochondrial transport and autophagy. Miro attaches mitochondria to the microtubule-based motor protein kinesin-1 and acts as a calcium-dependent switch for mitochondrial movement. Phosphorylation of Miro by Pink1 kinase and its subsequent Parkin-mediated degradation leads to mitophagy of damaged mitochondria. Relatively little is known about the molecular underpinnings of these processes and a structural understanding of the relevant protein machinery is lacking. Here we present the first crystal structures of human Miro1. The structures reveal a key nucleotide-sensing element and a Pink1 phosphorylation site both lie within an extensive EF hand/cGTPase interface. Interestingly, while SEC-MALS and SAXS studies show that full length Miro is monomeric, the isolated cGTPase domain dimerizes in solution. Crystal structures of the cGTPase dimer indicate that dimerization is mutually exclusive with the intramolecular EF hand/cGTPase interface and may have implications for Pink1-mediated recruitment of Parkin to the mitochondrial surface. Our results suggest structural mechanisms for phosphorylation-dependent regulation of mitochondrial function by Miro.

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Transition Metal FRET of Cyclic Nucleotide-Gated Channels Labeled with the Fluorescent Unnatural Amino Acid Anap
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Current methods are ineffective at measuring ion channel structural dynamics within a cellular membrane. Fluorescent labeling of proteins can report changes in protein structure, particularly when binding sites for transition metal ions are added near the fluorophore, because transition metal ions can quench fluorescence through a FRET mechanism (tmFRET). The use of small-molecule, cysteine-reactive fluorophores is limited in a cellular context, however, because background cysteine residues in biological membranes are also labeled, making changes in fluorescence difficult to interpret. As a result, unnatural amino acids with small fluorescent side chains are an appealing choice for specifically labeling ion channels expressed in cells. To this end, we incorporated a fluorescent unnatural amino acid, 3-(6-acetylnaphthalen-2-ylamino)-2-aminopropanoic acid (Anap) (Lee et al 2009; Chatterjee et al 2013), into the cyclic nucleotide-gated channel CNGA1 at position Q417 near the endogenous Ni^{2+}-potentiation site (H420) by replacing Q417 with an amber stop codon (CNGA1_Q417tag). Large currents were measurable from CNGA1_Q417tag channels in Xenopus ooctyes with both injection of a plasmid encoding orthogonal amber suppressor tRNA/aminoacyl-tRNA synthetase (pAnap) and incubation with L-Anap methyl ester. In the absence of either pAnap or L-Anap methyl ester, little or no current was observed, indicating that incorporation was specific for Anap at Q417tag. Compared to wild-type CNGA1 channels, CNGA1_Q417Anap channels had similar cyclic nucleotide- and voltage-dependence, and similar potentiation by both Ni^{2+} and Co^{2+} at H420. Since the absorption spectra of Co^{2+} overlaps substantially with the emission spectra of Anap, currents and fluorescence of CNGA1_Q417Anap channels were measured simultaneously using patch-clamp fluorometry with and without Co^{2+}. Anap fluorescence was quenched reversibly when Co^{2+} potentiated CNGA1_Q417Anap currents. This approach will be useful in measuring gating conformational changes of ion channel proteins with tmFRET.

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X-Ray Fluorescence Microscopy Analysis of Metal-Rich Inclusion Bodies in Adult Neural Stem Cells in Situ
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Imaging of metal distributions in rodent brains by X-ray fluorescence microscopy (XRF) revealed very concentrated (300 mM) localized (1 μm diameter) Cu deposits in periventricular neural stem cells. As it does not require alteration of the physiological metal concentration, XRF provides an ideal means to study the metal rich inclusion bodies in situ towards elucidating their biological function. It is first shown by XANES recorded from untreated tissue that Cu is bound as part of a multimetallic Cu-S cluster; imaging at 30 nm resolution reveals a consistent [S]/[Cu] ratio to be 1.66 ± 0.07. Accordingly, it is proposed that metallothionein (MT), a cysteine-rich protein known bind metals with high affinity as the binding protein. Parallel analysis of MT(1,2) knockout mice reveals that in the absence of MT(1,2), inclusions contain 40% less Cu while no other metals show significant variation anywhere else in the brain. Occurrence of inclusion bodies along the ventricle wall in MT(1,2)KO mice is three times lower in comparison with WT mice. Inclusions in mutants also exhibit indistinguishable XANES spectra and [S]/[Cu] ratios from wild-type controls. From neural stem cell basal bioavailability, these results suggest that MT3, a third MT isoform present only in the central nervous system, is capable of fulfilling the role of MT(1,2) in inclusion body formation. Finally, it is shown that inclusion bodies reside neither in lysosomes nor are they ubiquitinated. Periventricular astrocyte localization, morphology, and elemental composition suggest that these Cu bodies are homologous to previously reported Gomori-positive inclusion bodies which were thought to accumulate and gave rise to cell death via reactive oxygen species production in advanced age; their lack of involvement in an autophagy, however, suggests a new, currently unknown role for these Cu-rich inclusions.

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Revealing the Cellular Metabolic State through NADH Autofluorescence Lifetime in Parkinson's Disease
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Cellular metabolic imaging of the relative amounts of reduced NADH and the microenvironment of this metabolic electron carrier can be used to noninvasively monitor changes in cell metabolism in various diseases. Moreover, mitochondrial dysfunction, which is directly related to the cellular metabolic state, is considered to be central in developing Parkinson's disease (PD). We applied 2-photon fluorescence lifetime imaging microscopy to determine the fluorescence lifetime as well as the amounts of reduced NADH in PC12 cells (a cell line derived from pheochromocytoma of rat adrenal medulla) treated with Neuronal Growth Factor (NGF) to differentiate into neuronal cells. The neuronal cells were further treated with MPTP to induce PD syndromes and the change in fluorescence lifetime and the amounts of reduced NADH after the treatment were measured. Our preliminary results show a significant increase in the fluorescence lifetime of free as well as bound NADH in the PD induced cells. Moreover, the relative amounts of the ratio of free NADH over bound NADH does not show any significant difference among the control and PD cells. These results may lead to further understanding of the role of cellular metabolism in PD progression.