Synaptic Transmission

1371-Pos Board B215
Should a synapse be reliable?
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Synaptic transmission insures neuronal communication but relies on several random steps. Because synapse are still inaccessible to direct experimental recordings, to study synaptic reliability, we analyze synaptic transmission by constructing a biophysical model.
This model accounts for the synaptic cleft geometry and several dynamical variables such as the position of vesicular release and the membrane trafficking AMPA receptors, which mostly mediate the synaptic current. These receptors are located in the postsynaptic terminal, but can be exchanged from the Post-Synaptic Density (PSD), a fundamental microdomain and the extra-synaptic space.
We show that the synapse geometry controls the amplitude of the synaptic current, while receptor diffusional motion can replace desensitized receptors and thus prevents synaptic depression from receptor desensitization (significantly only after 6 to 7 successful spikes). Synaptic reliability is optimal when the active zone of vesicular release is apposed to a PSD, where AMPA receptors are concentrated. Change in this co-localization can lead to drastic effects on the synaptic current, which suggests that these changes can underlie a form of remodeling and plasticity. We finally demonstrate that fast temporal correlated spike lead to a reduced synaptic current. We conclude that although synapse should and are unreliable devices, at the neuronal level, reliability is restored due to the presence of multiple synaptic boutons.

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Molecular Dynamics Simulations Of Glutamate Diffusion In Membrane Bound Synaptic Cleft
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Diffusion of the transmitter in the synaptic cleft critically influences the amplitude and the time course of quantal events and thus strongly affects the synaptic efficacy. However, the value of the diffusion constant remains speculative. In a confined space diffusion of ions and molecules should in general be slower as they do not move exclusively in their solvated space, but also interact with walls of the synaptic cleft, which are fixed. Indeed molecular dynamics simulations demonstrated that the diffusion of glutamate- (and water) in the cleft formed by two single wall carbon sheets is slower, but only for clefts narrower than those of synapses in the central nervous system. To provide a more realistic assessment we simulate the diffusion of glutamate- (and water) in the cleft formed by lipid bilayers. The glutamate- molecules are layered near the bilayer, and partly interdigitate with it, and the interfacial glutamate–bilayer interactions modulate the speed of glutamate diffusion. Water molecules also interdigitate with the bilayer, which masks the layering near the bilayer. The diffusion of glutamate and water in the cleft bound by lipid bilayers is influenced by factors similar to those observed in the cleft bound by carbon sheets - cleft separation and charge on the atoms of the wall. Finally, the movement of atoms of the lipid bilayer (evaluated by 'freezing' the positions of all atoms of the bilayer) also affects the diffusion of glutamate- and water. In conclusion this study provides a more realistic evaluation of spatial distribution and diffusion of glutamate- and water in the synaptic cleft and how they are influenced by the interactions with the membrane.

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The SNARE-binding Protein Complexin Modulates the Kinetics of Neurotransmitter Release and Short-term Synaptic Plasticity
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Neurotransmitter release requires calcium-dependent synaptic vesicle fusion mediated by Synaptotagmin 1 and the SNARE complex. Complexin is a synaptic binding partner of the neuronal SNARE complex and has been implicated as an effector of vesicle priming and fusion, and as a synaptic vesicle fusion clamp. However, the precise physiological role of complexin in vesicle fusion and short-term synaptic plasticity is unknown. Here, we investigate the role of complexin in neurotransmitter release and short-term synaptic plasticity at the *Drosophila* neuromuscular junction (NMJ). We present a thorough analysis of synaptic transmission in complexin null mutants and complexin overexpression strains using voltage-clamp recordings at the NMJ. Kinetic analysis of evoked current reveals that synchronous and asynchronous release depends critically on the level of complexin expression. Additionally, complexin regulates early short-term synaptic depression and facilitation in opposite fashion, through modulation of release probability and the immediately releasable pool. We propose that complexin decreases the free energy of SNARE complex-mediated priming and generates an energetic barrier at a late stage by clamping vesicles in the immediately releasable pool.

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Synaptotagmin's Role as the Ca2+ Sensor in Regulated Exocytosis
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Exocytosis of neurotransmitters is triggered by the initial influx of Ca2+. Synaptotagmin I is known to bind Ca2+ and the phospholipid membrane to modulate this process. The mechanism, however, for this information transduction is not well known. We seek to understand how this information is conveyed through the protein. A single point mutation, Y180F, has been made to correlate with a mutant that has been seen in vivo to display diminished physiological function. This mutant will be utilized as a probe as we seek to ascertain the mechanism of the signal transduction. The Y180F mutation is located in the binding pocket of the first C2 domain of synaptotagmin. It was hypothesized that this mutation abolishes the hydrogen bonding potential between this position and His237 and that the lack of hydrogen bonding will lead to a drastic decrease in stability in the binding pocket. This mutation is predicted to manifest itself as a reduction in the domain's calcium ion affinity. To test these predictions, Ca2+ and phospholipid binding assays for wild type C2A and Y180F will be carried out and monitored via steady state fluorescence as well as protein denaturation assays. Partition functions will be derived to quantify the results so a thermodynamic comparison can be carried out.

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A Physiological And Molecular Biological Study Of Rat Cerebellar Long-term Depression
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The synaptic plasticity is thought to be one of the most important physiological substrates for memory and learning. In order to investigate the possible role of gamma 7 (TARP family protein) at the synapse between parallel fibers and Purkinje cells which causes synaptic plasticity (long-term depression, LTD) as the basis of motor learning, we tried to isolate and clarify the sequence of gamma 7 gene taken from rat cerebellum. We succeded to identify the gamma 7 gene. Moreover, patch clamp electrophysiological measurements were carried out from rat cerelellar acute slice preparations by using UV-laser beam (351, 364 nm) for uncaging of MNI-caged glutamate to stimulate dendritic trees of Purkinje cells. We compared the evoked responses by photolysis with those obtained from electrical stimulation. The time course and amplitude in these respenses evoked and photolysis were almost equal. The possible involvement of the TARP in the regulation of synaptic transmission in Purkinje cell is investigated together with photolysis experiment in the present research.

Membrane Transporters & Exchangers I

1376-Pos Board B220
Molecular Mechanisms of Cl^-/H^+ Coupling in CLC-ec1
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The bacterial CLC-ec1 is a structurally known H^+/Cl^- exchanger of the CLC family and it has served as an excellent model to predict many functional properties of the eukaryotic CLC channels and transporters. We investigated two fundamental questions in the mechanism regulated coupled Cl^-/H^+ exchange: first, is H^+ movement rate limiting? Second, what are the molecular determinants of Cl^-/H^+ coupling in CLC-ec1?
To investigate whether H^+ movement is rate or not in the transport cycle we measured the isotope effect associated with exchanging H^+ with D^+ on the transport rate. We found that this substitution has little effect on the Cl^- transport rate, suggesting that H^+ binding or its movement across the membrane are not rate limiting. This implies that either Cl^-binding or a conformational change in the protein is the rate limiting step.
We then studied the coupling between H^+ and Cl^- binding. If n H^+ bind to or are released by CLC-ec1 in response to a Cl^- binding event then the enthalpy of Cl^- binding to CLC-ec1 measured with isothermal titration calorimetry, ΔH_{tot}, is the sum of two components: Cl^- binds to CLC-ec1 and n H^+ are released into or absorbed from the surrounding solution, ΔH^o_{bind}, and subsequently binds to