

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.

1372-Pos Board B216

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.

1373-Pos Board B217

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.

1374-Pos Board B218

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.

1375-Pos Board B219

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 succeeded to identify the gamma 7 gene. Moreover, patch clamp electrophysiological measurements were carried out from rat cerebellar 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 responses 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_{bind}^o, and subsequently binds to

a buffer molecule, ΔH_{buf} . By measuring the enthalpy in several buffers we can determine the number of H^+ binding or unbinding from CLC-ec1 in response to Cl^- binding. We found that for WT CLC-ec1 $n \sim 0.5$, suggesting that for every 2 Cl^- binding 1 H^+ is released. This is in agreement with the transport stoichiometry of CLC-ec1. This binding coupling is ablated by mutations that inhibit H^+ transport. Neutralization of either of the two H^+ -accepting glutamates of CLC-ec1 leads to impairment in Cl^- -coupled H^+ release.

1377-Pos Board B221

Mutation on the External Gate Changes the pH-dependency of a CLC Cl^-/H^+ Exchanger

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The CLC-ec1 is a bacterial homologue of the transporter-type CLC subclass that catalyzes transmembrane exchange of Cl^- and H^+ . Glu_{ex} (E148 in CLC-ec1) is the pH-dependent external gate of both Cl^- and H^+ transport. The central tyrosine, Y445 (Tyr_{cen}) coordinates the central Cl^- ion and acts as the internal gate of the Cl^- pathway. Glu_{in} (E203) is remotely located to the internal Cl^- gate and acts as the internal proton-transfer residue that delivers protons from the internal aqueous solution to the protein interior. Wild type CLC-ec1 has the pH-dependent Cl^- flux: as the pH increased, the transport rate of Cl^- is decreased. It has been shown that mutations on the Glu_{ex} lead to the loss of pH-dependency of Cl^- flux while on the Glu_{in} retain relatively intact pH-dependency. We therefore examined mutations of Glu_{ex} and their functional consequences on the pH-dependency. Mutations with neutral amino acids, A or Q (E148A or E148Q) completely lose H^+ movement as well as pH-dependency of Cl^- transport. E148H mutant also fail to transport H^+ , but strikingly reverses pH-dependent transport of Cl^- , as does the doubly mutated transporter, E148H/E203H. These data support the idea that the pH-dependency is mainly came from the Glu_{ex} not Glu_{in} . Currently, we are investigating the effect of other mutants having various ranges of pKa value and their structural and functional consequences.

1378-Pos Board B222

Measuring CIC-Transporters on Solid Supported Membranes

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Members of the family of CIC-chloride channels and transporters have received increasing attention in the last years because of their important physiological functions and their implication in pathogenesis. Some are important targets for drug discovery while others (e.g. CIC-7) are still poorly investigated due to the lack of suitable electrophysiological methods.

Recently, electrophysiological measurements based on solid supported membranes (SSM) have been used for the functional characterization of ion pumps and transporters. In this technique proteoliposomes, membrane vesicles or membrane fragments are adsorbed to a SSM and are activated by rapid substrate concentration jump. Then charge translocation is measured via capacitive coupling to the supporting membrane.

SSM-based electrophysiology is extremely useful in cases where conventional electrophysiology cannot be applied. Apart from a few rare exceptions bacterial transporters cannot be investigated using voltage clamp or patch clamp methods and also mammalian transporters predominantly expressed in intracellular compartments are not accessible for standard electrophysiology.

Here we apply SSM-based techniques to two proteins of the CIC-family which function as H^+/Cl^- exchangers: the bacterial CIC-ec and the putative transporter CIC-7 from lysosomes. CIC-ec was purified from *E. coli* and reconstituted into liposomes. The proteoliposomes were adsorbed to the SSM and transient currents were measured after activation of the protein with a Cl^- concentration jump. This allows time resolved measurements of charge translocation and kinetic analysis of the transport mechanism. CIC-7 was investigated using a stable expressing CHO cell line. Cell membranes were prepared and adsorbed to the SSM. Also CIC-7 was activated using a Cl^- concentration jump and transient currents were recorded. This allows the first electrophysiological characterization of CIC-7 and its functional analysis.

1379-Pos Board B223

Functional Characterization Of Clc-5 Mutations Associated With Dent's Disease

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The Cl^-/H^+ antiporter CIC-5 has been linked to Dent's disease, an X-linked renal disease associated with low molecular weight proteinuria, hypercalciuria and nephrolithiasis. CIC-5 is expressed on early endosomes of proximal tubule

cells, where it plays a critical role in endosomal function. The impact of Dent's disease-causing mutations on CIC-5 function has not been yet fully investigated. Here, we have analysed an unpublished mutation K115R and three published mutations, Y272C, N340K and K546E in terms of electrical activity and trafficking at the plasma membrane in *Xenopus laevis* oocytes. A construct carrying an extracellular HA epitope (kindly provided by T. J. Jentsch, MDC/FMP, Berlin) that does not alter the CIC-5 wild-type (WT) currents allowed us to evaluate surface expression of the different CIC-5 using a chemiluminescence test. The currents were measured by two-electrode voltage-clamp. The mutant K115R induced a reduction of $68 \pm 1.9\%$ of WT CIC-5 currents ($p < 0.001$, $n=17$). Currents recorded with Y272C ($n=6$), N340K ($n=7$) and K546E ($n=6$) mutants were not significantly different from non-injected oocytes. The loss of currents for the mutants N340K and K546E correlated well with a loss of surface expression: chemiluminescence signals were not significantly different from those observed in non-injected oocytes ($p < 0.001$, $n=6$). We found no significant difference between surface expression of K115R ($n=5$), Y272C ($n=4$) and WT CIC-5. In conclusion, N340K and K546E mutants have a defective targeting to the oocyte plasma membrane, and K115R and Y272C a reduced electrical activity. Further studies should investigate whether the targeting to early endosomes is faulty in the case of the first type of mutations and how the regulation or the conduction pathway are involved in the case of the second type of mutations.

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Binding Sequence And Coupling Of H^+ And Na^+ Ions In The Glutamate Transporter (glt)

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Substrate transport in GIT is catalyzed by co-transport of three Na^+ (Na_1 , Na_2 and Na_3) and one H^+ , and counter-transport of one K^+ . The crystal structure of GIT, however, provides information only about the position of the substrate and two Na^+ ions. In order to investigate the transport cycle in GIT, therefore, the first step is to identify the binding sites for the H^+ and for the third Na^+ ion. Careful examination of the structure in the context of the possible transport pathway indicates that there are only two titratable acidic residues (Asp312 and Asp405) that might serve as the H^+ binding site during the transport cycle. We have performed a set of MD simulations (30 ns each) with different combinations of substrate and protonation states of Asp405 and Asp312. Our simulations show that water has no access to Asp312, but can easily reach Asp405 in the apo state, suggesting that Asp405 is possibly the first H^+ binding site. The access of Asp312 to the extracellular solution is blocked by an H-bond network between Asn310, Asn401, and Asp405. Interestingly, protonation of Asp405 results in disruption of this network allowing access of water to Asp312. In search for the third Na^+ binding site, a water molecule near Asp312 (a putative Na^+ binding site) was randomly replaced by a Na^+ at the beginning of four independent simulations. The placed Na^+ was observed to move into and be stabilized by a binding site formed by Asp312, Thr92, Asn310 and Ser93. Based on these results, we propose that H^+ binding precedes that of Na_3 , as protonation of Asp405 is required for hydration of Asp312 and providing access for Na^+ binding.

1381-Pos Board B225

Exploring the Gating Mechanism of the Glutamate Transporter GltPh

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Glutamate acts as the primary neurotransmitter in the mammalian central nervous system. Clearance of this neurotransmitter from the synapse is accomplished by a family of glutamate transporters known as EAATs. These proteins move synaptic glutamate across the cell membrane into the cell against its concentration gradient. It has long been speculated that the mechanism for transport involves the movement of extracellular and intracellular gates, providing "alternating access" to a substrate binding site. Recently, the crystal structure of a related bacterial transporter, GltPh, was solved, revealing two helical hairpins (HP1 and HP2) which have been proposed to contribute to these gates. HP2 lies on the extracellular face of the transporter, and a number of studies have shown that this hairpin can adopt multiple conformations that either provide or restrict access to the substrate binding site. However, to date there is no structural information regarding the conformational changes that must occur within HP1 in order to provide the substrate a path to the intracellular solution. Here we use the technique of site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy to explore the structure of HP1 (residues 258-290) in purified GltPh reconstituted into proteoliposomes. This technique provides an opportunity to probe the dynamic structure of HP1 in a functional protein embedded in a physiological lipid environment.