

a simple model for the reversal of the CPX clamp by Synaptotagmin and calcium. In this model, Synaptotagmin binds the stalled CPX-SNARE complex via its interactions with the t-SNAREs and upon binding Calcium, the membrane loops inserts rapidly into the bilayers, while Synaptotagmin stays bound in place on the SNAREpins. This is expected to perturb the attached SNAREpin out of the planarity of the zigzag array and thus, trigger the rapid disassembly of the stalled zigzag structure allowing the SNAREs to complete zippering and open a fusion pore.

2546-Pos Board B565

The Dorsal Root Ganglion Sandwich Synapse: Novel Transglial Signaling between Neuronal Somata

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The dorsal root ganglion (DRG) contains a subset of closely-apposed neuronal somata (*NS*) that are separated solely by a thin satellite glial cell (*SGC*) membrane septum to form a *NS-glial cell-NS (NGIN)* cell trimer. We recently reported that stimulation of one *NS* evokes a delayed, noisy and long-duration inward current in both itself and its passive partner that was blocked by suramin, a general purinergic antagonist. Here we test the hypothesis that *NGIN* transmission involves purinergic activation of the *SGC* and its release of an excitatory transmitter. Stimulation of the *NS* triggered a sustained current noise in the *SGC*. Block of transmission through the *NGIN* by reactive blue 2 or thapsigargin, a Ca^{2+} store-depletion agent, implicated a Ca^{2+} store discharge-linked P2Y receptor. P2Y2 was identified by simulation of the *NGIN*-like transmission by puff of UTP onto the *SGC*. Block of the UTP effect by BAPTA, an intracellular Ca^{2+} scavenger, supported the involvement of *SGC* Ca^{2+} stores in the signaling pathway. The response to UTP was also blocked by AP5, which, along with the N2B subunit-specific antagonist ifenprodil, inhibited *NGIN* transmission, implicating a glutamatergic pathway via postsynaptic NMDA receptors. Puff of glutamate could evoke transmission-like current in the *NS*. Immunocytochemistry localized the NMDA receptor subunit NR2B to the *NS* membrane, abutting staining for P2Y2 on the *SGC* septum. We infer that *NGIN* transmission involves secretion of ATP from the *NS*, *SGC* Ca^{2+} store discharge via P2Y2 receptors and release of glutamate to activate *NS* postsynaptic NMDA receptors. Thus, the *NS* of the *NGIN* trimer communicate via a "Sandwich Synapse" transglial pathway, a novel signaling mechanism that may contribute to information transfer in other regions of the nervous system.

2547-Pos Board B566

The Interactions of Dopamine and L-Dopa with Lipid Headgroup and its Implication for Neurotransmitters Metabolism

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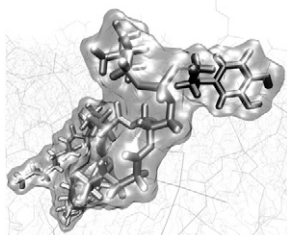
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Atomistic molecular dynamics simulations were used to investigate the interactions between the neurotransmitter dopamine and its precursor L-dopa with membrane lipids. The results indicate that these molecules strongly interact with the lipid head groups e.g. via hydrogen bonds. These interactions anchor the dopamine and L-dopa to the membrane interfacial region. The strength of this bonding is dependent on lipids present in membrane. Presence of phosphatidylserine resulted in increased level of bonding strength with a lifetime longer than the timescale of our simulations.

The high membrane association of dopamine and L-dopa both, extracellularly, might favour the availability of these compounds for cell membrane uptake processes and, intracellularly, can accentuate the importance of membrane-bound metabolizing enzymes over their soluble counterparts.

We can also hypothesize that excessive association of dopamine and its precursor L-dopa with the membranes in the situation, when the concentration of phosphatidylserine is increased, may eventually limit the free use of dopamine as a synaptic transmitter what could possibly be a molecular level mechanism responsible for some of neurodegenerative disorders.



2548-Pos Board B567

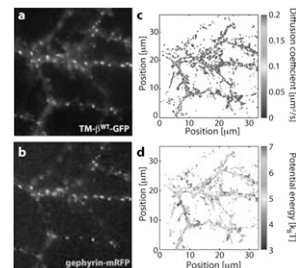
Mapping the Energy Landscapes of the Glycine Receptor in the Post-Synaptic Neuronal Membrane

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The movement of proteins in the cell membrane is governed by the local friction and their interactions with molecular partners. Yet, most experimental descriptions fail to unequivocally distinguish these effects; instead, they combine the diffusive and energetic contributions into an effective diffusion coefficient or anomalous exponent. Here, we show how the diffusion and energy landscapes of membrane proteins can be mapped separately over the entire cell surface using high-density single-molecule imaging and statistical inference [1]. In the case of glycine neuroreceptors, we demonstrate that scaffolds at inhibitory synapses act as energy traps with a depth modulated by the properties of the intracellular loop that mediates the receptor-scaffold interactions. Furthermore, we bridge the gap between local properties of the membrane environment and characteristics of the mobility at the cellular scale by simulating random walks in the inferred maps and computing estimators such as the propagator, mean square displacement, and first-passage time. Results are used to investigate the relation between numbers of receptors and synaptic plasticity. Overall, our approach provides a versatile framework with which to analyze biochemical interactions in situ.

[1] J.-B. Masson et al, Nat. Chem. (submitted)



2549-Pos Board B568

High Precision Release of Neurotransmitter - A New Tool

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Modern imaging techniques have become a powerful tool for investigating excitation patterns and signaling pathways in the brain. However, the high complexity of brain topology and the resolution limit of *in vivo* techniques make it difficult to study isolated small neuron circuits. Micro-electrode arrays (MEAs) are able to record activity of *in vitro* neuron networks and to stimulate locally, but such electrical approach cannot easily be applied to stimulate single cells. Large stimulus artifacts and poor control over the spread of electrical stimuli in medium create the main disadvantages when studying the role of single cells in small networks. One promising solution is to interact with individual neural cells by mimicking chemical signaling. Recently developed systems for precise neurotransmitter release include micropipettes, microfluidic substrates and caged compounds.

In this project we propose to combine MEA technology (Multi Channel Systems GmbH, Switzerland) with novel FluidFM technology. The FluidFM (Cytosurge AG, Switzerland) has hollow atomic-force microscopy (AFM) cantilevers acting as force-controlled nanopipettes. We present the ability to locally release neurotransmitters on the cell membrane with precise control over applied force (sub-nN) and spatial position (μm). For those experiments we used FluidFM cantilevers with $2 \mu\text{m}$ openings, where the microfluidic channel was filled with physiological solution containing $200 \mu\text{M}$ glutamate. In order to chemically induce a local electrical response in a culture of dissociated hippocampal neurons from E18 rat, we first brought the FluidFM cantilever in contact with the cell membrane. We then applied pressure-pulses between 50 mbar and 300 mbar with 300 ms duration to locally eject sub picoliter volumes of the neurotransmitter.

We are now working towards applying single cell stimulations to well defined networks.

2550-Pos Board B569

Cocaine Preferentially Potentiates Fast Releasable Vesicle Pool in Mouse Dopaminergic Striatum *In Vivo*

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Cocaine, an addictive drug, increases extracellular dopamine (DA) concentration in basal ganglion. This effect has been commonly assumed mainly through the inhibition of the uptake activity of DA transporters (DAT), although other mechanisms such as cocaine potentiation of DA release is less examined under physiological conditions. Here we report that two kinetically distinct DA releasable vesicle pools, a fast releasable pool (FRP) and a prolonged releasable pool (PRP), existed following physiological field stimulations at medial fore-brain bundle in mouse striatum *in vivo*. Surprisingly, cocaine selectively enhanced the DA release and replenishment from the FRP. Using a cocaine-insensitive-DAT mouse model, cocaine-facilitated FRP (not PRP) release is mediated by DAT. In contrast, PRP (not FRP) release is selectively sensitive to D2 receptor (D2R). Thus, in addition to blocking DA reuptake, cocaine increases DA release through selectively activating DAT, increasing FRP size, and accelerating FRP replenishment in mouse striatum *in vivo*.

2551-Pos Board B570

Development of an Activity Dependent, Photochemically-Released Tool for the Study of Calcium Permeable AMPA Receptors

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The ability to observe the movement of neurotransmitter receptors in and around a synapse could provide crucial new information to our understanding of synaptic plasticity, the process that likely underlies memory formation. We have developed a new class of fluorescent probes designed to target fluorophores to natively-expressed neuronal receptors. This strategy allows for receptors to be covalently-tagged and tracked in a non-perturbed state; thus allowing for visualization of complex neuronal processes. Specifically, calcium-permeable, non-NMDA glutamate receptors (CP-AMPA) expressed in hippocampal neurons can be targeted with this novel tri-functional molecule. CP-AMPA have recently been shown to play a role in some forms of synaptic plasticity, aiding such processes as long-term potentiation and depression, but their basal location around the synapse remains unknown. In brief, our molecular design employs a use-dependent polyamine ligand which targets only the receptors receiving glutamatergic input at the time of labeling, a promiscuous electrophile for covalent bond formation with a nucleophilic sidechain amino acid on the channel, and a fluorophore for visualization. Bioconjugation of this molecule results in stable covalent bond formation between the probe and the target receptor. An additional aspect of our first generation probes is that the ligand is connected to the remainder of the probe with a photolabile linker, thus allowing the receptor to re-enter the non-liganded and native state.

2552-Pos Board B571

Synaptic Vesicles Isolated from the Electric Organ of Torpedo Californica Posses Multiple Classes of Neurotransmitter Transporters

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The fusion of synaptic vesicles with the presynaptic membrane, and the subsequent release of small chemical neurotransmitters is the fundamental process by which neurons communicate at chemical synapses, and it has long been proposed that most neurons release a single type of small molecule neurotransmitter. The simplicity of one neuron, one neurotransmitter has come under intense scrutiny as examples of neurons that appear to co-release two or more neurotransmitters at single synapses (neurotransmitter synergy) have been identified. One such synapse is that of the electric organ of *Torpedo californica*; synaptic vesicles isolated from this single class of neurons appear to contain the neurotransmitter acetylcholine as well as the neurotransmitter ATP. We used immunofluorescence labeling in conjunction with single-molecule TIRF microscopy to observe whether one or more neurotransmitter transporters could be detected on single synaptic vesicles. We found that the vesicular acetylcholine transporter (VACHT) and several solute carrier proteins (SLC17A) co-localized to the same vesicles. The presence of multiple types of neurotransmitter transporters - and potentially neurotransmitters - in individual synaptic vesicles raises fundamental questions about chemical synaptic transmission at the electric organ of *T. californica*. In addition, this approach can be applied to other synapses in order to address the prevalence of neurotransmitter synergy and co-release at chemical synapses.

2553-Pos Board B572

Alpha2Delta Calcium Channel Subunit Constrains Steep Dependence of Release Probability on Calcium Channel Density through Coupling to Kv1.1 at Nerve Terminals

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Exocytosis of neurotransmitter at synapses is steeply dependent on the concentration of Ca^{2+} in the vicinity of the Ca^{2+} sensor of exocytosis. The abundance of Ca^{2+} channels in the active zone is expected to exert a powerful influence on release probability as each additional channel at the active zone is expected to increase Ca^{2+} influx as well as decreases the average distance between sites of Ca^{2+} entry and sites of exocytosis. As this spatial coupling is also highly non-linear, modest changes in Ca^{2+} channel numbers at active zones would potentially drive synapses into maximal release conditions. Calcium influx at nerve terminals is also regulated by the action potential waveform, which dictates the channel open probability and driving force. Using the latest generation of genetically encoded voltage-indicators, we show here that the clinically important and rate-limiting determinant of calcium channel abundance at nerve terminals, the voltage-gated Ca^{2+} channel subunit $\alpha 2\delta$ also controls the shape of local action potential waveform in the presynaptic terminal. Overexpression of $\alpha 2\delta$ in hippocampal neurons narrows the action potential waveforms and accentuates the hyperpolarization in en passant boutons while ablation of $\alpha 2\delta$ via shRNA-mediated knockdown both broadens the waveform and eliminates the hyperpolarization phase. These shifts in waveform were no longer detectable when *Shaker* potassium channels (Kv1.1 channels) were blocked pharmacologically by either tetrodotoxin or dendrotoxin-K. Our data imply that in addition to controlling local calcium channel abundance at nerve terminals $\alpha 2\delta$ also utilizes Kv1.1 voltage-gated potassium channels to sculpt the action potential. We believe that this provides an important control for synapses with high release probability by preventing potentially dangerous levels $[Ca^{2+}]_i$ from entering through higher numbers of voltage gated calcium channels during stimulation.

2554-Pos Board B573

Effects of Isoflurane on Intracellular Calcium in Boutons of Hippocampal Neurons

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Current knowledge of the molecular and cellular mechanisms of anesthetic action is insufficient to explain amnesia, unconsciousness or immobilization, the principal features of general anesthesia. A more detailed knowledge of the blocking mechanisms of volatile anesthetics (VAs) on presynaptic targets is critical to understanding their synaptic mechanisms of action. Here we show inhibition of synaptic vesicle (SV) exocytosis by the volatile anesthetic isoflurane as a function of nerve terminal $[Ca^{2+}]_i$, determined in the presence of varying external $[Ca^{2+}]_o$ to test the hypothesis that VAs inhibit neurotransmitter release upstream of Ca^{2+} entry and thus reduce exocytosis in proportion to reductions in intracellular $[Ca^{2+}]_i$. Laser-scanning fluorescence image measurements of intracellular Ca^{2+} in transfected neonatal rat hippocampal neurons were made using the genetically encoded Ca^{2+} -indicator GCaMP3 (Tian *et al.*, 2009) or the acetoxymethyl (AM) ester of the Ca^{2+} -sensitive dye fluo5 (10 μ M) (Ariel & Ryan, 2010), and action potential (AP)-evoked exocytosis was measured with synaptophysin-pHluorin. The effect of isoflurane on $[Ca^{2+}]_i$ correlates with its effect on exocytosis at varying external $[Ca^{2+}]_o$ to reveal minimal direct effect on the Ca^{2+} sensitivity of exocytosis. These data suggest the primary anesthetic targets in the inhibition of neurotransmitter release from small synaptic vesicles are situated upstream of Ca^{2+} entry and not within the synaptic vesicle fusion machinery.

2555-Pos Board B574

Complexin I Plays a Bilateral Role in Synaptic Transmission during Development at the Calyx of Held Synapse

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Complexins are small synaptic proteins which cooperate with the SNARE-complex during synaptic transmission. Different roles of complexins in the regulation of vesicle exocytosis have been proposed. Based on the results of genetic mutation or knock down/out studies, it is generally agreed that complexins are involved in vesicle priming and exocytosis during fast synchronous release and in clamping vesicles to prevent asynchronous release. However, depending on cell type, organism and experimental approach used, complexins appear to either facilitate or inhibit vesicle fusion. Here, we study the function of complexin I at the calyx of Held synapse. By taking advantage of the large size of the calyx terminal, allowing direct patch-clamp recordings, we investigate the consequences of the loss of function of complexin I. We demonstrate a developmentally aggravating phenotype of reduced EPSC amplitudes and enhanced asynchronous release. We provide evidence for a role of CPX I in recruiting Ca^{2+} channels to docked vesicles which may determine their release