

membrane capacitance measurement. By recording vesicle fusion with capacitance measurements at this synapse, here we show that acute application of BDNF inhibits exocytosis. This effect is specific to BDNF because application of K252a, an inhibitor of the BDNF receptor, TrkB, blocks the actions of BDNF on presynaptic function. Moreover, BDNF inhibits rapid and slow endocytosis, thus providing a regulatory mechanism for vesicle recycling. Furthermore, we recorded the presynaptic calcium current directly at the calyx nerve terminal and found for the first time that BDNF inhibits calcium current. It has been shown previously, that both exo- and endocytosis are regulated by calcium influx. Thus, our findings establish that neurotrophins regulate the function of the nerve terminal via presynaptic calcium channels. Further understanding of these mechanisms will advance our knowledge of synaptic transmission regulation, a process essential for our brain function.

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Synapses between Interneurons in the Rat Cerebral Cortex at 2NM Resolution and in Three-Dimensions

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We used thin sectioning and freeze-fracture electron microscopy to study the synapses in the rat cerebral cortex at 2nm resolution and in three-dimensions. The aim was to determine whether the opening of vesicles in the pre-synaptic axon was correlated to the distribution of the neurotransmitter receptors in the post-synaptic spine predicted by the long-term potentiation (LTP) of synaptic strength hypothesis. In the axon, "hybrid" filaments assembled from rods protruding from the vesicle and the plasma membrane docked synaptic vesicles to the active zone. The orientation of the "hybrid" filaments with respect to the plasma membrane defined two vesicle-docking paths. When parallel to the plasma membrane, the vesicles fuse "randomly" on the active zone, a pathway that was predicted by the SNARE hypothesis. When perpendicular to the plasma membrane, the vesicles dock at the base of plasma membrane depressions (35-45 nm), which are spaced 75-85 nm center-to-center, arranged in files and contained within gutters (the "deterministic" path). This path was not anticipated by the SNARE hypothesis. Computer simulations indicate that the "deterministic" path creates neurotransmitter concentration domains on the post-synaptic spine that are wider and shallower than those of the "random" path. In both paths, the opening of the fusion pore follows steps anticipated by the fusion-through-hemifusion hypothesis. In the post-synaptic spine, we identified intra-membranous particles in the external (E) face with dimensions that match the homotetrameric GluR2 AMPA-sensitive receptor. The particles are either clustered on the E face or distributed in bands on the "non-synaptic" plasma membrane. We thus conclude that in the pre-synaptic axon vesicle exocytosis follows "random" and "deterministic" fusion paths while in the post-synaptic spine LTP of synaptic strength involves the rearrangement of pre-existing receptors instead of de novo insertion and retrieval.

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Syp1 Acts as a Clearance Factor for Syb2 at the Presynapse

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Fusion of synaptic vesicles (SVs) during synaptic transmission is mediated by SNARE complex assembly formed by coiled-coiling of the SNARE proteins synaptobrevin2 (Syb2), syntaxin-1A and SNAP-25. In order to maintain neurotransmission, exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. Clathrin-mediated endocytosis (CME) is thought to be the predominant mechanism. However, it might be too slow for fast SV recycling. Therefore, a pre-sorted and pre-assembled surface pool of SV proteins at the presynapse, the 'readily retrievable pool' (RRetP), might support a first wave of fast CME.

Using fluorescence nanoscopy of labeled SV proteins in combination with quantitative k-nearest neighbour and paired correlation analysis, we were able to resolve the spatial distribution of the surface pool at the presynaptic membrane and identified dimerization of Syb2 as a first important step in self-assembly of surface nanodomains. Moreover, we show that the SV protein synaptophysin1 (Syp1) coordinates and sequesters these dimers in larger nanodomains containing a few ten molecules of both Syb2 and Syp1. Both, dimerization and co-expression of Syp1 are sufficient for correct targeting of Syb2 to presynaptic structures. These results were confirmed using shRNA-mediated knockdown of Syp1 or Syp1 knockout mice. Using pHluorin-fusion constructs expressed in hippocampal neurons, we show that dimerization of Syb2 is necessary for proper sorting into endocytosing SVs, since Syb2 monomers stranded at the plasma membrane cause frequency-dependent short term depression (STD) as a result of compromised Syb2 clearance away from release sites.

This finding is corroborated in Syp1 knockout neurons that display strong frequency-dependent STD. Our results demonstrate the functional role of the Syb2-Syp1 interaction in sequestering Syb2 into the RRetP for efficient release site clearance, thereby preventing cis-SNARE complex formation.

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Single Molecule Motion Maps of Open and Desensitization States of Nicotinic Acetylcholine Receptors

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The nicotinic acetylcholine receptor (nAChR) is one of the most thoroughly studied neurotransmitter receptors belonging to a pentameric ligand-gated ion channel (pLGIC) family. Physiological analyses show that there are several states for nAChRs, such as open, resting, intermediate desensitization, or chronically desensitized states, however the structural information of each state was not fully solved yet, and mechanism of changing states remain incompletely understood. We have addressed to elucidate dynamic information of nAChRs in different states by single molecule technique with diffracted X-ray tracking (DXT). We had previously reported that DXT could trace functional motion of protein machinery at single molecule level in tilting and twisting rotational axes views (1,2). Gold nanocrystal immobilized on a protein is used as tracer for structural change of the target protein. We adapted this method for nAChR and compared the two-rotational motion map of α -subunits nAChR in the presence of acetylcholine (ACh), α -bungarotoxin (α Btx), or excess of nicotine. We found that the combined tilting and twisting motions of α -subunit nAChR were enhanced with ACh, those motions were inhibited with α Btx, and only tilting motion was inhibited with excess nicotine in the solution. (Twisting motion is existing even in desensitized state.) A recent electron crystallography study demonstrated that ACh binding to the α -subunit of nAChR triggered a distortion in the ligand-binding domain [3], which involved the outward displacement of the extracellular portion of the β subunit. The finding is consistent with our result that ACh enhances the motion of the ligand-binding domains of nAChR. DXT is highly precise and has the ability to detect important intra-molecular motions of channel proteins.

[1] Shimizu et al, Cell 132:67 (2008).

[2] Sekiguchi et al, PLOS ONE 8:e64176 (2013).

[3] Unwin & Fujiyoshi, JMB 422:617 (2012).

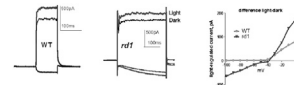
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Photoswitching HCN Channels in Degenerated Retina Neurons

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Some degenerative retinal diseases involve death of rods and cones, while the remaining retinal cells remain intact, yet nonresponsive to light. An azobenzene-photoswitch ion-channel-*trans*-blocker DENAQ restores retina light sensitivity in mouse model of photoreceptor degeneration (*rd1*), without affecting wild type (WT) animals. We compared ion currents in retinal ganglion cells (RGCs) of the WT and *rd1* mice, and their regulation by light following application of DENAQ. Voltage-gated K-currents were regulated by DENAQ similarly in RGCs of the WT and *rd1*-retinas (19% vs 21%-increase by 480nm-light). However, DENAQ photosensitized hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels in *rd1* RGCs, but not WT retinas. The WT-RGCs revealed mainly fast-activating (tens ms) I_h , while most *rd1*-RGCs showed a substantial portion of slow I_h ($\tau_a > 100$ ms). Illumination produced 21%-increase of I_h (1.3pA/pF at -100mV) within hundreds ms in DENAQ-pretreated (150 μ M) neurons. Same light in DENAQ-treated WT-RGCs produced no fast changes, while some neurons showed small slow decrease of I_h (<5% developing within 5sec). We hypothesize that slow HCN channels up-regulated in degenerated retina, might be more sensitive to DENAQ than the fast ones in the WT, but do not exclude other targets mediating light-effects on I_h .



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Development of a Red Genetically-Encoded Voltage Indicator and its use with Channelrhodopsin for All-Optical Electrophysiology

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The need for effective genetically-encoded voltage indicators is widely recognized. Several prototypes have been made to date by fusing one or more