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1620-Pos Board B390
Calcium-Induced Calcium Release (CICR) Triggers Fusion of Individual Synaptic Vesicles in Rod Terminals
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Photoreceptor light responses are encoded by changes in synaptic vesicle release. Release from rods is triggered by the opening of calcium channels beneath plate-like synaptic ribbons. Maintained depolarization can activate CICR and enhance release. Using total internal reflection fluorescence microscopy (TIRFM) to visualize release of single synaptic vesicles, we tested whether CICR enhances release from rods by stimulating fusion at non-ribbon sites.
Rods from salamander retina were loaded with activity-dependent dyes, FM1-43 or dextran-conjugated pHrodo, and visualized by TIRFM. Rods were depolarized with steps to −10 mV under voltage-clamp or by puff application of 50 mM KCl. CICR was activated with 10 μM ryanodine and inhibited with 100 μM ryanodine. Ribbon locations were identified with a fluorescent ribbon-binding peptide or from hot spots of depolarization-evoked calcium entry visualized with Fluo5F.
In terminals loaded with FM1-43 or pHrodo, depolarization stimulated rapid disappearance of vesicles with kinetics similar to that measured electrophysiologically. Additionally, stimulation-evoked vesicle disappearance was blocked by Cd^{2+}, indicating that it was due to calcium-dependent exocytosis. Vesicles docked for about 200 ms before fusion. Most release events occurred close to ribbons, but some also occurred further away. Activation of CICR with 10 μM ryanodine stimulated intracellular calcium increases and vesicle release. Ryanodine-evoked release events were less clustered than release evoked by depolarization, consistent with greater non-ribbon release. The spread of calcium evoked by 500 ms steps (but not 50 ms steps) was inhibited by blocking CICR with 100 μM ryanodine in the patch pipette. Release evoked by 500 ms steps also involved sites further from the ribbon than release evoked by 50 ms steps. These results indicate that the stimulation of CICR by maintained depolarization enhances release from rods by triggering fusion of vesicles at non-ribbon sites.

1621-Pos Board B391
The Role of Mobile Calcium Buffers in Synaptic Transmission at the Inner Hair Cell Ribbon Synapse
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Temporally precise sound encoding at the inner hair cell (IHC) ribbon synapse is tightly regulated by calcium. The mobile calcium buffers calbindin, parvalbumin alpha and calretinin might contribute to shaping the presynaptic Ca^{2+} signals. We investigated the function of these calcium binding proteins in IHC synaptic transmission by examining the auditory phenotype of double and triple buffer knockout mice. Our results show that buffer deficiency does not significantly alter hearing thresholds; however, we observed a slight increase in peak and steady-state sound-driven spike rates of spiral ganglion nerve fibers in knockout mice. The presynaptic function of IHCs was first studied by perforated patch-clamp recordings of Ca^{2+} currents and exocytic membrane capacitance increments. The absence of mobile calcium buffering proteins augmented sustained exocytosis in IHCs while leaving the amplitude and kinetics of exocytosis of the readily-releasable pool unchanged. Further, Ca^{2+}-dependent inactivation of calcium currents was stronger in IHCs of triple buffer knockout mice. In ruptured patch experiments we then tried to restore the calcium buffer capacity by adding exogeneous buffers. We estimated the concentration of endogenous buffers in IHCs to be equivalent to 0.5-1 mM BAPTA, which agrees well with previous estimates obtained by quantitative immunogold electron microscopy (Hackney et al., 2005). Our results demonstrate that calbindin, parvalbumin alpha and calretinin are involved in the regulation of synaptic transmission at the IHC ribbon synapse; however they do not seem to be essential for hearing, at least not during transient sound stimulation.

1622-Pos Board B392
Action Potential-Triggered Somatic Exocytosis in Mesencephalic Trigeminal Nucleus Neurons in Rat Brain Slices
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The neurons in the mesencephalic trigeminal nucleus (MeV) play essential roles in proprioceptive sensation of the face and oral cavity. The somata of MeV neurons are generally assumed to carry out neuronal functions but not to play a direct role in synaptic transmission. Using whole-cell recording and membrane capacitance (Cm) measurements, we found that the somata of MeV neurons underwent robust exocytosis (Cm jumps) upon depolarization and with the normal firing of action potentials in brain slices. Both removing $[Ca^{2+}]_o$ and buffering $[Ca^{2+}]_i$ with BAPTA blocked this exocytosis, indicating that it was completely Ca^{2+}-dependent. In addition, an electron microscopic study showed synaptic-like vesicles approximated to the plasma membrane in somata. There was a single Ca^{2+}-dependent releasable vesicle pool with a peak release rate of 1912 fF/s. Importantly, following depolarization-induced somatic exocytosis, GABA-mediated postsynaptic currents were transiently reduced by 31%, suggesting that the somatic vesicular release had a retrograde effect on afferent GABAergic transmission. These results provide strong evidence that the somata of MeV neurons undergo robust somatic secretion and may play a crucial role in bidirectional communication between somata and their synaptic inputs in the central nervous system.

1623-Pos Board B393
Calcein Inhibits Vesicle Release
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Charged fluorescent dyes are frequently used as fluid phase makers to detect vesicle fusion events with the cell membrane by means of transient omega-like indentations. We used calcein (300 μM) to image vesicle fusion from nerve growth factor (NGF)-treated PC12 cells, a model secretory cell line. We stimulated the cells with either high K^+ saline or 100 μM nicotine to evoke calcium-dependent vesicle secretion. However, when the cells were stimulated in the presence of calcein, they showed a reduction in the number of vesicles that were released and/or endocytosed compared to cells that were stimulated in the absence of calcein. This observation from imaging experiments led us to hypothesize that calcein was inhibiting vesicle release from NGF-treated PC12 cells. In order to understand whether calcein was having an inhibitory affect on vesicle secretion and/or reuptake of vesicle membranes, we utilized amperometry to analyze vesicle release of catecholamine transmitter content from NGF-treated PC12 cells. Amperometry is an electrochemical detection method used routinely to measure release of vesicle contents from individual cells with a carbon fiber electrode. Analysis of amperometric spikes provides information as to the number of catecholamine molecules released and the kinetics of release. Our results show that calcein caused a reduction in the number of released amperometric spike events from 106 ± 13 events measured from control nicotine-stimulated cells, to 60 ± 11 events ($p<0.05$) in the presence of calcein. Peak amplitude, half-width, quantal content, or kinetics of rising or falling phase were unaffected by calcein. These data indicate that calcein has an inhibitory effect on vesicle fusion with the cellular membrane resulting in reduced amperometry events and a subsequent reduction in uptake of the fluid phase marker when used to track vesicles while imaging.

1624-Pos Board B394
Exocytic Mechanisms of Storage and Release of Brevetoxin in the Dinoflagellate Karenia Brevis
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Karenia brevis is a broadly distributed toxic dinoflagellate responsible for Red Tide outbreaks throughout the world. Deleterious effects of these blooms are caused by brevetoxin, a potent neurotoxin that binds to sodium channels in nerve and muscle cell leading to substantial marine life mortality and human