Thursday, May 20, 13.00, at the BBB, Auditorium 4

Visualization of long-term plasticity of vesicular transmitter release using two-photon laser scanning microscopy

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Long-term potentiation (LTP) and depression (LTD) are believed to be important mechanisms of information storage, learning, and memory. While postsynaptic alterations associated with long-term synaptic plasticity have been richly studied, studies of presynaptic plasticity have waxed and waned as the mistaken "pre *versus* post" arguments have raged, and as access to small CNS presynaptic terminals has made it difficult to directly measure vesicular transmitter release. Over the past few years, we exploited the spatial excitation resolution of multi-photon laser scanning microscopy to overcome background fluorescence in using FM1-43 to visualize release in brain slices with more intact synaptic networks.

Using these methods, we have shown that a component of LTD is due to long-term reduction in vesicular release probability, while LTP is associated with long-term increases. Recently, we combined electrophysiology and multi-photon imaging to measure the mode of vesicular release and exchange rates between vesicle pools before and after inducing LTD in acute hippocampal slices from mice expressing the pH-sensitive eGFP-tagged SNARE protein synaptopHluorin (SpH). We compared the balance of exocytosis and endocytosis during low-frequency (2Hz) and high-frequency (20Hz) stimulation by measuring SpH fluorescence increases produced by synaptic stimulation in slices before and after treatment with the vacuolar ATPase inhibitor Bafilomycin (Baf, 1µM) to isolate rates of exocytosis by alkaline trapping of released vesicles. By comparing ratios of endo/exocytosis at 2Hz (0.86) and 20Hz (0.43), we conclude there is a significant "kiss-and-run" contribution to transmission that is larger during low frequency transmission, because more kiss-and-run release allows endocytosis rates to keep up with exocytosis.

Evidence suggests that presynaptic inhibition mediated by G protein-coupled receptors (GPCR) involves a direct interaction between G proteins and the vesicle fusion machinery. In particular, the C terminus of the SNARE protein SNAP-25 is a molecular binding target of Gβγ liberated by activation of presynaptic GCPR, leading us to test whether this interaction is necessary for LTD. We found that the C terminus of SNAP-25 is a necessary moiety for the expression of LTD, but not LTP, of synaptic strength at Schaffer collateral-CA1 synapses in the hippocampus. Using type A botulinum toxin (BotoxA) to enzymatically cleave the 9 amino acid C-terminus of SNAP-25, we found that BotoxA eliminated the ability of low frequency synaptic stimulation to induce LTD, but not LTP, even if release probability was restored to pre-BotoxA levels by raising extracellular $[Ca^{2+}]_o$. Electroporation of CA3 pyramidal neuron somata to presynaptically infuse the 14-amino acid C-terminus of SNAP-25 (Ct-SNAP-25) as a scavenger of Gβγ, reduced both the transient presynaptic inhibition produced by the metabotropic glutamate receptor agonist DCG-IV, and also LTD. Thus, the C-terminus of SNAP-25, which links synaptotagmin I to the SNARE complex, appears to be a target for Gβγ necessary for both transient transmitter-mediated presynaptic inhibition, and for the induction of longer-lasting LTD.

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