Delivering the goods

Activity-induced exocytosis and AMPA receptor insertion within a post-synaptic membrane compartment depends on syntaxin 4

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The activity-dependent strengthening of transmission at individual synapses has long been postulated to underlie learning and memory in the brain, and current wisdom strongly suggests that molecular modifications within both the pre- and post-synaptic nerve terminals contribute to this strengthening process (i.e. long-term potentiation or LTP). At excitatory, glutamatergic synapses, the dynamic insertion and retrieval of ionotropic glutamate receptors into and from the post-synaptic plasma membrane have been implicated in synaptic plasticity, however, the site(s) for these trafficking events and the molecules involved have not be clearly elucidated. Biochemical studies have identified SNARE proteins as critical mediators of membrane targeting and fusion events in many cell types, including neurons, and several bacterial toxins are known to interfere with neurotransmission by disrupting the function of membrane-bound SNARE proteins, such as VAMP and syntaxin. Using high resolution imaging techniques, the authors of this study have characterized the molecular processes underlying activity-driven membrane fusion events within dendritic spines, tiny membrane protrusions only 1–2 microns in size. Their data demonstrate that syntaxin 4 functions as a key SNARE protein for the exocytic insertion of glutamate receptors and the membrane trafficking events contributing to synaptic plasticity in dendritic spines.

To examine the movement of glutamate receptors from the endocytic compartment to the plasma membrane of dendritic shafts and spines, the authors took advantage of the well characterized recycling behavior of the transferrin receptor. Once this cell surface receptor is internalized with its cargo of iron-bound transferrin, it is processed through endosomes and then recycled to the plasma membrane as an empty receptor. Exploiting this natural trafficking movement, the authors created a real-time optical reporter of protein movement between endocytic vesicles and the plasma membrane by fusing red fluorescent protein (mCherry) to the extracellular domain of the transferrin receptor (TfR), along with a pH-sensitive variant of green fluorescent protein (i.e. super-ecliptic pHluorin, SEP). Once expressed in transfected neurons, this TfR-mCh-SEP reporter would allow detection of the entire dendritic pool of transferrin receptor by monitoring red fluorescence, and selective detection of plasma membrane-localized transferrin receptor by the appearance of green fluorescence. Typically, the combined red and green fluorescence of surface localized TfR-mCh-SEP would appear as yellow fluorescence. Following internalization of the TfR-mCh-SEP reporter via endocytosis, the low acidity of endocytic vesicles (i.e., pH 5–6) would quench the green fluorescence of the fused SEP, but not affect the mCherry fluorescence. Endocytosed transferrin receptors that are recycled to the plasma membrane would be exposed to the neutral pH of the extracellular solution, thereby permitting SEP to fluoresce. Simultaneous imaging of the red and green fluorescent signals of the TfR-mCh-SEP reporter thus allowed the authors to follow exocytic processes before, during and after synaptic stimulation.

When transiently transfected into cultured hippocampal neurons isolated
from E18 embryonic rats, the TfR-mCh-SEP reporter was observed intracellularly in both dendritic shafts and spines, and appeared to remain within these structures for tens of minutes under basal conditions. Exposure of transfected neurons to fluorescent transferrin protein led to uptake and labeling of intracellular TfR-mCh-SEP-expressing vesicles, indicating that the TfR-mCh-SEP protein in neurons was indeed functional. By labeling endogenous cell surface AMPA-type glutamate receptors with an antibody directed against an extracellular epitope of the GluR1 subunit, the authors further observed that 85% of dendritic spines expressing TfR-mCh-SEP also contained internalized AMPA receptors, indicating that endogenous post-synaptic density proteins may also traffic via an endocytic pathway in dendritic spines. Similar results were obtained for exogenously expressed GluR1 receptors with SEP fused to the extracellular domain.

Exposure of TfR-mCh-SEP transfected neurons to a cocktail designed to evoke synaptic activity (i.e., 0 Mg²⁺, glycine, bicuculline) stimulated fusion of endosomal compartments with the plasma membrane at the level of dendritic spines. Furthermore, the rapid appearance of SEP fluorescence at the surface of dendritic spines (i.e., time constant of 0.6 sec) occurred adjacent to the edge of the post-synaptic density (i.e., within 300 nm). Following stimulation of exocytosis in the spine, TfR-mCh-SEP associated fluorescence tended to slowly diffuse out of the spine and accumulate in the dendritic shaft. In contrast, the stimulated insertion of SEP-tagged GluR1 receptors into the spine plasma membrane appeared to occur in an “all-or-none” manner suggestive of complete fusion of endosomal vesicles. Moreover, nearly two-thirds of the newly inserted receptors at the fusion site lateral to the post-synaptic density (PSD) appeared to be stably incorporated or retained at this exocytic location.

In the pre-synaptic terminal, syntaxin 1 functions as a critical t-SNARE protein for the engagement and fusion of exocytic vesicles, however, the functionally equivalent syntaxin isoform in the post-synaptic dendritic compartment has not been clearly identified. In the present study, immunocytochemical labeling of hippocampal neurons revealed enriched, punctate staining of syntaxin 4 (Stx4) in the plasma membrane of dendritic spines that was localized adjacent to PSDs. In contrast, the synaptic distributions of other plasma membrane-localized syntaxin isoforms (i.e., Stx1, 2 and 3) were either predominately pre-synaptic or displayed similar intensities pre- and post-synaptically. Importantly, the authors observed that exocytosis of internalized TfR-SEP proteins in transfected neurons occurred primarily at Stx4-enriched sites near the PSD of dendritic spines, strongly suggesting that Stx4 defines a SNARE-mediated zone for rapid exocytosis within spines. To test this hypothesis, the authors performed shRNA-based knock down of endogenous Stx4 protein (i.e., to 25–30% of normal cellular levels) and observed that activity-evoked exocytosis of endosomal TfR-SEP was nearly abolished within dendritic spines. Similar interference of exocytosis was observed when TfR-SEP was co-expressed with a dominant-negative, soluble form of Stx4 (i.e., one lacking the transmembrane segment, but still capable of associating with endogenous cognate SNARE proteins, such as VAMP and SNAP-25). As a control, soluble forms of either Stx2 or 3 did not have similar inhibitory effects, in line with the authors’ earlier data suggesting that Stx4 acts as the major t-SNARE for the fusion of recycling endosomes in dendritic spines. This dominant-negative strategy was further utilized to examine the role of Stx4 in stimulus-induced LTP. Following introduction of soluble Stx4 into neurons via a recording patch pipette, the authors observed that stimulus-evoked LTP was almost abolished, whereas a soluble form of Stx3 had no effect on this form of synaptic plasticity.

Collectively, these findings demonstrate that synaptic activity promotes the fusion of endosomal vesicles in dendritic spines, leading to the insertion of AMPA-type glutamate receptors near the post-synaptic density. Stx4 appears to be a critical t-SNARE in the plasma membrane of dendritic spines for this activity-evoked exocytosis in spines, and disrupting its function interferes with both membrane fusion and the strengthening of synaptic transmission via LTP. Exactly how newly inserted AMPA receptors are selectively retained in the dendritic spine, compared to exocytosed transferrin receptors that diffuse to sites within the dendritic shaft, was not described, but may depend upon accessory subunits and/or scaffolding proteins that promote incorporation of AMPA receptors within the PSD. The results of this study thus identify one molecular mechanism underlying the dynamic remodeling of post-synaptic membranes in the CNS.