Generally Physiological

Of transmission at unconventional synapses

This month's installment of *Generally Physiological* considers co-release of antagonistic neurotransmitters and insights into vesicle replenishment and the mechanism of neurotransmitter release at ribbon synapses.

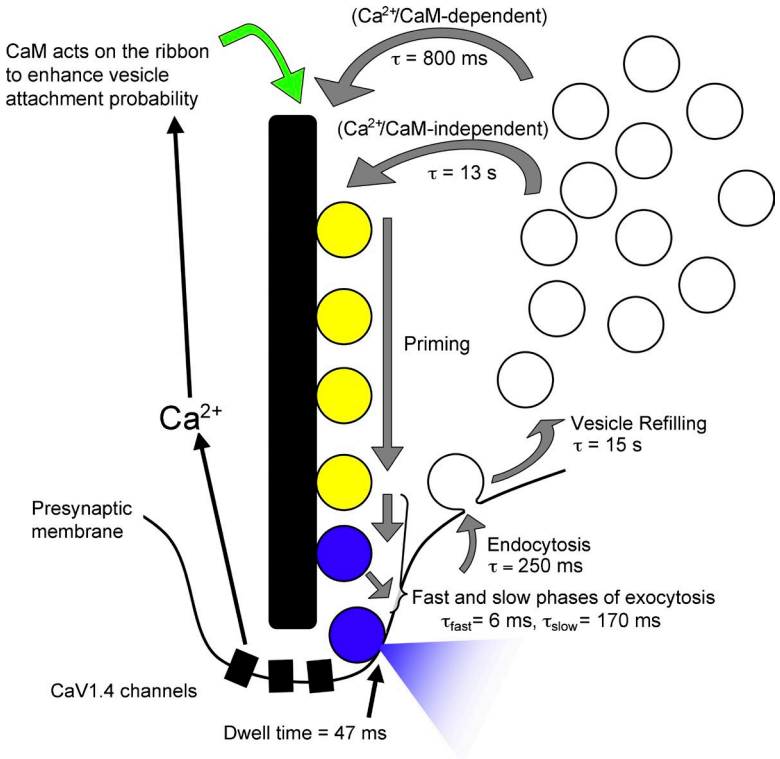
Co-releasing glutamate and GABA

Brain function depends on the appropriate balance between excitatory and inhibitory signals, with glutamate acting as the most common excitatory neurotransmitter and GABA (γ-aminobutyric acid) acting as the most common inhibitory neurotransmitter. Typically, inhibitory signaling mediated by GABAergic interneurons counterbalances glutamatergic excitation. However, two studies from different research groups used a combination of approaches, including electrophysiology, optogenetics, and immunohistochemistry, to identify neurons from the basal ganglia that co-release GABA and glutamate onto individual neurons in the lateral habenula (Root et al., 2014; Shabel et al., 2014), a brain region lacking GABAergic interneurons.

Both groups concluded that GABA and glutamate were present in the same nerve terminals, with Shabel et al. (2014) providing evidence from immunogold labeling consistent with colocalization of the two transmitters in individual vesicles. Root et al. (2014), who studied co-transmission from neurons from the ventral tegmental area, observed complex patterns of response to in vivo optical stimulation of the co-releasing neurons. With stimulus parameters designed to evoke burst firing (10-ms pulses of light presented every 2 s), they observed that a majority of spontaneously active habenular neurons showed inhibition (which could be followed by excitation) and a minority showed excitation (which could be followed by inhibition). Using pharmacological analysis of cell-attached recordings in brain slices, Shabel et al. (2014), who investigated co-transmission from entopeduncular neurons, determined that GABA had a greater effect on limiting habenular firing rate during high-frequency than low-frequency stimulation. Intriguingly, the latter group also found that the ratio of GABA- to glutamate-mediated synaptic responses was lower in a rat model of depression and higher after chronic treatment with a serotonin-based antidepressant, suggesting that manipulation of GABA/glutamate co-transmission could potentially provide a new approach to treating mood disorders.

Accelerating ribbon replenishment

Neurotransmitter release from vertebrate photoreceptors, like that from auditory and vestibular hair cells and various other cells mediating early stages of sensory processing, is



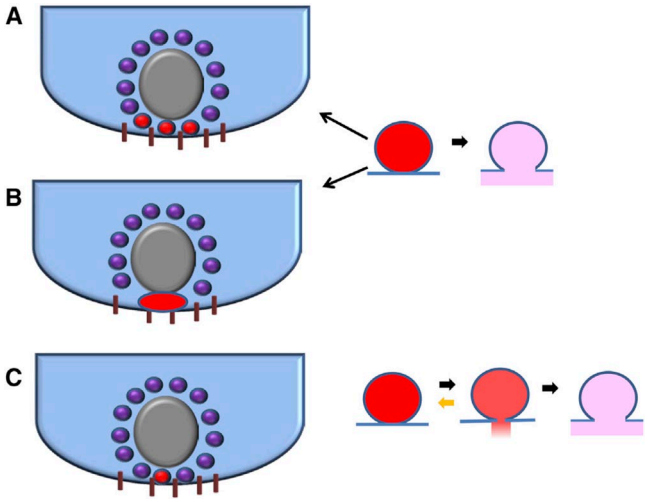
Proposed model for the vesicle cycle at the ribbon synapse of cone photoreceptors. Calcium acts through calmodulin to enhance a fast mechanism whereby vesicles attach to the ribbon and thereby accelerates replenishment. A much slower process of vesicle attachment is calcium-calmodulin independent. Blue vesicles represent the immediately releasable pool, responsible for the fast phase of exocytosis, and yellow vesicles represent a ribbon-associated reserve pool. (From Van Hook et al., 2014.)

ongoing, with the rate of release modulated by graded changes in membrane potential. These tonically active synapses are characterized by a specialized structure, the synaptic ribbon, which provides a continuous supply of primed vesicles ready for release at the active zone. In this issue, Van Hook et al. explored the mechanism of calcium-mediated acceleration of vesicle replenishment at tiger salamander cone ribbon synapses and its role in visual perception. A combination of electrophysiology, total internal reflection fluorescence microscopy, and modeling indicated that calcium acts through calmodulin to promote vesicle attachment to the ribbon, thereby augmenting the faster of two kinetically distinguishable components of vesicle replenishment and enabling the transmission of higher-frequency information from cones to subsequent neurons in the visual processing pathway.

Variability of individual hair cell release events

Ribbon synapses are also characterized by marked heterogeneity of the amplitude of individual excitatory postsynaptic currents (EPSCs). This variability has been attributed to multivesicular release, occurring through either the simultaneous fusion of multiple vesicles with the plasma membrane or the homotypic fusion of multiple synaptic vesicles before exocytosis (see Lv and Zenisek [2014]). However, Chapochnikov et al. (2014) propose that, at rat inner hair cell ribbon synapses, large multiphasic EPSCs are uniquantal—arising from transient openings of a dynamic fusion pore—rather than secondary to multiquantal release. Noting that many multiphasic EPSCs show "foot events," similar to those associated with release from nascent fusion pores in adrenal chromaffin cells, Chapochnikov et al. (2014) compared the distributions of EPSC charge and EPSC amplitude. Charge showed less variability and a more symmetric (single peak) distribution than did amplitude, consistent with release from a single vesicle (uniquantal release); moreover, the mean charge of monophasic EPSCs was comparable with that of multiphasic EPSCs, suggesting that they were triggered by the same amount of neurotransmitter. Multiphasic EPSCs persisted in the absence of calcium influx (inconsistent with their arising from calcium-dependent coordination of multivesicular release through a shared nanodomain surrounding an open channel), although the fraction of monophasic EPSCs increased (compatible with calcium regulation of the fusion pore). Functional modeling in combination with ultrastructural data were inconsistent with the homotypic fusion model of release, whereas modeling indicated that a dynamic fusion pore—together with the large ring-like clusters AMPA receptors present postsynaptically—could account for the variability of EPSC size and shape. The authors thus propose that, at the rat inner hair cell ribbon synapse, EPSC heterogeneity may arise from uniquantal release through a flickering fusion pore.

Co-release of antagonistic neurotransmitters and insights into vesicle replenishment and the mechanism of neurotransmitter release at ribbon synapses



Models for heterogeneity of ribbon synapse EPSC amplitude. (A) Simultaneous release of multiple vesicles. (B) Homotypic fusion of synaptic vesicles. (C) Flickering of the fusion pore of a single vesicle. (Reprinted from *Neuron*, 83, C. Lv and D. Zenisek, Big Minis from Hair Cells: Mechanism and Function, 1229–1231, 2014, with permission from Elsevier.)

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REFERENCES

Chapochnikov, N.M., et al. 2014. *Neuron.* 83:1389–1403. http://dx.doi.org/10.1016/j.neuron.2014.08.003

Lv, C., and D. Zenisek. 2014. *Neuron.* 83:1229–1231. http://dx.doi.org/10.1016/j.neuron.2014.09.013

Root, D.H., et al. 2014. *Nat. Neurosci.* In press. http://dx.doi.org/10.1038/nn.3823

Shabel, S.J., et al. 2014. *Science.* 345:1494–1498. http://dx.doi.org/10.1126/science.1250469

Van Hook, M.J., et al. 2014. *J. Gen. Physiol.* 144:357–378. http://dx.doi.org/10.1085/jgp.201411229