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Review

The Nanophysiology of Fast Transmitter Release

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Action potentials invading the presynaptic terminal trigger discharge of docked synaptic vesicles (SVs) by opening voltage-dependent calcium channels (CaVs) and admitting calcium ions (Ca^{2+}), which diffuse to, and activate, SV sensors. At most synapses, SV sensors and CaVs are sufficiently close that release is gated by individual CaV Ca^{2+} nanodomains centered on the channel mouth. Other synapses gate SV release with extensive Ca^{2+} microdomains summed from many, more distant CaVs. We review the experimental preparations, theories, and methods that provided principles of release nanophysiology and highlight expansion of the field into synaptic diversity and modifications of release gating for specific synaptic demands. Specializations in domain gating may adapt the terminal for roles in development, transmission of rapid impulse frequencies, and modulation of synaptic strength.

Early Studies on the Transmitter Release Mechanism

The seminal experiment demonstrating that transmitter release from presynaptic terminals requires both simultaneous nerve terminal depolarization and the presence of extracellular Ca^{2+} [1] was the lifetime favorite (personal communication) of Sir Bernard Katz (Nobel Laureate in Physiology and Medicine, 1970). This key observation, together with the findings that depolarization opens presynaptic **CaVs** (see Glossary) [2] admitting Ca^{2+} into the nerve terminal cytoplasm [3] and that intracellular Ca^{2+} can gate transmitter release [4] by the fusion of SVs [5] with the surface membrane, formed the basis of the 'calcium hypothesis' of transmitter release gating. The essential link between influx of Ca^{2+} through the channel and its subsequent binding to the **SV sensor** was not discussed in Katz's monograph and is the subject of this review. Several previous reviews [6–9] provide additional perspectives on this subject.

Exemplar Presynaptic Experimental Preparations

Because the vast majority of presynaptic terminals are small and inaccessible, progress in this field has relied heavily on a relatively few exemplar model synapses and the application of remarkably innovative experimental assay methods.

Squid Giant Synapse (SGS)

The SGS (Figure 1A) was the first synapse at which it was possible to record directly from the presynaptic terminal [10]. Application of a two- or three-electrode voltage clamp (Figure 1A, lower panel) [2,11] heralded the modern era in synaptic research, relating presynaptic inward Ca^{2+} current to transmitter output. Three findings at the SGS were of particular significance with respect to release gating. First, a minimum latency of 0.2 ms between Ca^{2+} influx through the CaVs and transmitter release [12] implied that at least some CaVs must lie within ~100 nm from the SV sensors. Second, the demonstration that, typically, transmitter release was maximal during the repolarization phase of the action potential [13] permitted calculation of realistic Ca^{2+} influx rates; and third, the finding by the Charlton group that fast- but not slow-binding

Trends

Single domain gating appears to predominate where transmission fidelity is paramount [73]. Possible reasons include: minimal delay due to the virtually instantaneous access of Ca^{2+} to the SV sensor; protection against docked-SV depletion and transmission failure; a metabolic advantage by minimizing Ca^{2+} clearance; and avoiding detrimental effects of high cytoplasmic Ca^{2+} [8].

Overlapping domain gating may predominate where the amplitude of transmitter release is more important than the frequency of impulses [76] or where release is subject to modulation of presynaptic CaVs. While rod synapses contradict this general idea, since they exhibit an exquisite dynamic range while relying on single domain gating [68], it is possible that this idea holds for neurons.

Single domain-type synapses may pass through a pre-maturity overlapping domain stage, raising the possibility that amplitude coding is important during synapse formation [60].

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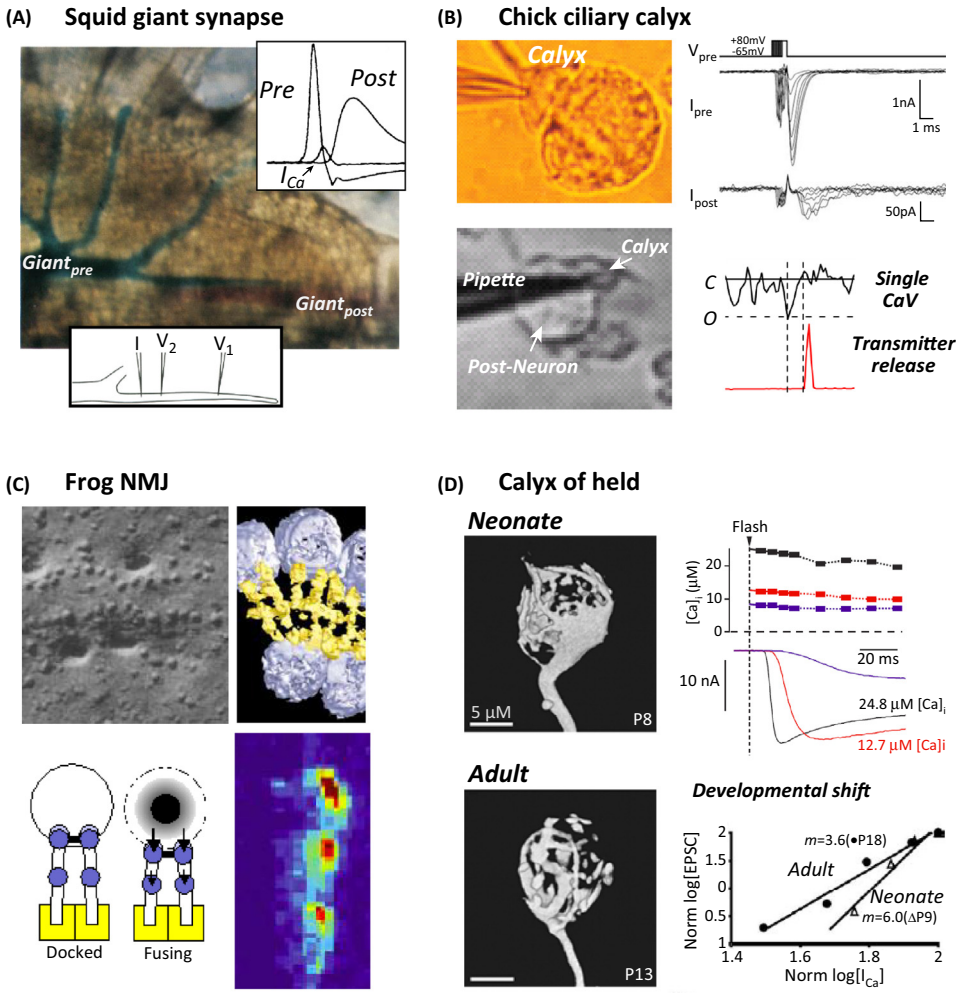

Figure 1. Exemplar Presynaptic Terminals. Experimental investigation directly on the presynaptic terminal is difficult mostly because of small size, inaccessibility, or heterogeneity and hence much of what we know about the general principles of synaptic transmission physiology derives from exemplar experimental models. (A) Squid giant synapse (SGS). The giant synapse is embedded in the stellate ganglion of the squid where a presynaptic giant axon from the optic ganglion synapses onto a giant axon in each of the mantle nerve bundles. The contact from the presynaptic giant onto the postsynaptic giant axon in the last mantle bundle is termed the SGS (see lower inset) [10]. In the figure the presynaptic giant axon has been filled with dye [11]. Ca^{2+} gating of transmitter release was analyzed by voltage clamp of the presynaptic terminal using two or three (as shown, lower inset) sharp electrodes while also recording from the post-synaptic neuron. Right inset: The presynaptic calcium current (I_{Ca}) evoked by an action potential-simulated voltage command (Pre) triggers transmitter release detected as an excitatory postsynaptic current (Post) [13]. (B) Chick ciliary ganglion calyx synapse (chick CC). The presynaptic terminal at calyx-type synapses envelopes the postsynaptic neuron with a sheet-like or reticulated membrane process [79]. The chick CC was isolated either attached to (upper-left panel) [80,81] or partially removed from (lower-left panel) [16] the postsynaptic neuron. Double whole-cell patch clamp recording (upper-right panel) demonstrated a near-single-power relationship between the presynaptic I_{Ca} (I_{pre}) and the postsy-naptic current (I_{post}), favoring single domain gated release (Box 1) [15]. Cell-attached patch recording of single CaV activity was conducted on partially isolated calyces [16] while monitoring transmitter release (lower-right panel). The finding that transmitter release is linked to individual Ca^{2+} current flickers argued that fusion of a synaptic vesicle (SV) was gated by the opening of a single presynaptic voltage-dependent calcium channel (CaV) [17]. O, open; C, closed single-channel current level. (C) Frog neuromuscular junction. Freeze-fracture replicas of the transmitter-release face of actively secreting terminals exhibit two pairs of large particle rows bordered by SV fusion profiles (top-left panel) [82]. A putative scaffold linking the SVs to the particles has been imaged by electron microscopy tomography [83] and evidence suggests that the structure contracts during exocytosis [64] (top-right and bottom-left panels, respectively). Action potentials in the nerve terminal evoke discrete Ca^{2+} plumes, as detected by fluorescent dye, corresponding to the opening of individual CaVs (lower-right panel) [25]. (D) Neonatal calyx of Held (nCoH) and adult CoH. The rodent CoH undergoes increased

Glossary

Microdomain: a high-concentration plume of ions in the cytoplasm resulting from the overlap of nanodomains from a cluster of open membrane channels. The size and concentration profile of microdomains can be predicted to be variable as they are affected by the number of channels in the cluster, their spatial relationship, and their individual open–closed fluctuations.

Nanodomain: a high-concentration plume of ions in the cytoplasm resulting from the opening of a single ion channel. The nanodomain forms and collapses virtually instantaneously on channel opening and closing and can be predicted to be relatively consistent in profile for given cell conditions.

Nanophysiology: a new term coined to include processes operating at submicron distances where function must ultimately be related to constraints on individual molecules.

Overlapping domain gating: activation of the SV sensor by Ca^{2+} entering through more than one surface membrane CaV.

Ribbon synapse: a transmitter release site common in sensory cells characterized by an electron-dense structure that abuts the SV fusion region.

Single domain gating: activation of the SV sensor by Ca^{2+} entering through a single surface membrane CaV.

SV sensor: the molecular apparatus associated with the docked SV responsible for binding multiple Ca^{2+} ions and thence gating SV fusion. The SV sensor includes synaptotoagmin-1 but may require other ion-binding or function-translating molecules.

Voltage-dependent calcium channel (CaV): there are three families of CaVs: CaV1, CaV2, and CaV3. Of these, CaV2.1 and CaV2.2 are the principle types involved in gating SV fusion at fast-transmitting synapses but CaV2.3 can also play a role. However, CaV1-family channels are preferred at sensory receptor ribbon-type synapses and CaV3 channels can also gate release at a few synaptic contacts.