Presynaptic calcium and control of vesicle fusion
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Vesicle fusion and transmitter release at synapses is driven by a highly localized Ca\(^{2+}\) signal that rapidly builds up around open Ca\(^{2+}\)-channels at and near presynaptic active zones. It has been difficult to estimate the amplitude and the kinetics of this ‘microdomain’ signal by direct Ca\(^{2+}\)-imaging approaches.

Recently, Ca\(^{2+}\) uncaging at large CNS synapses, among them the calyx of Held, has shown that the intrinsic cooperativity of Ca\(^{2+}\) in inducing vesicle fusion is high, with 4–5 Ca\(^{2+}\) ions needed to trigger vesicle fusion. Given the Ca\(^{2+}\)-sensitivity of vesicle fusion as determined by Ca\(^{2+}\)-uncaging, it was found that a surprisingly small (10–25 \(\mu\)M) and brief (< 1 ms) local Ca\(^{2+}\) signal is sufficient to achieve the amount, and the kinetics of the physiological transmitter release. The high cooperativity of Ca\(^{2+}\) in inducing vesicle fusion and the non-saturation of the Ca\(^{2+}\)-sensor for vesicle fusion renders small changes of the local Ca\(^{2+}\)-signal highly effective in changing the release probability, an insight that is important for our understanding of short-term modulation of synaptic strength.

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Introduction
Information transfer between neurons takes place at highly specialized contact sites, the synapses. On the presynaptic side, the active zone harbors docked, readily releasable vesicles. It also contains a cytoskeletal matrix [1], which defines the sites of synaptic vesicle fusion in molecular terms. Ultrastructural analysis using electron microscopy techniques has shown that active zones are generally small, with an area of around 0.1 \(\mu\)m\(^2\) for various CNS excitatory synapses [2–4]. Active zones are thought to be the sites of Ca\(^{2+}\)-influx and of fusion of readily releasable vesicles upon arrival of an action potential (AP) at the nerve terminal. This review summarizes recent work on the Ca\(^{2+}\)-dependent mechanisms of phasic transmitter release at CNS synapses, thus focusing on the Ca\(^{2+}\)-triggering of readily-releasable vesicles. A more complete description of the presynaptic vesicle cycle can be found in recent reviews [5,6]. Work on synaptotagmin, a candidate protein for the presynaptic Ca\(^{2+}\)-sensor for vesicle fusion, has been summarized elsewhere [7,8].

The intracellular Ca\(^{2+}\) signal that triggers vesicle fusion
Ultsrastructural information about the exact location of Ca\(^{2+}\) channels at and around presynaptic active zones is sparse for CNS synapses. The concept that local, microdomain Ca\(^{2+}\) signals are responsible for triggering vesicle fusion mainly evolved from theoretical analyses and from functional studies. Computer simulations in the 1980s and early 1990s showed that the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) rises and falls very rapidly in the immediate vicinity of Ca\(^{2+}\)-channels when they open and close. Thus, the rapid rise and drop of transmitter release probability during a presynaptic AP is best explained by a tight, tens of nanometer range co-localization between docked vesicles and Ca\(^{2+}\)-entry points [9–12]. We will call the intracellular Ca\(^{2+}\) signal ‘seen’ by an average docked and fusion competent vesicle in a given secretory cell or nerve terminal the ‘local’ [Ca\(^{2+}\)], in contrast to the global, spatially averaged [Ca\(^{2+}\)], which can be measured by Ca\(^{2+}\)-indicator dyes.

Evidence for the microdomain nature of the local [Ca\(^{2+}\)] signal has also been obtained from functional studies that employ fast imaging techniques with submicrometer resolution. Imaging of a low-affinity variant of the luminescent Ca\(^{2+}\)-indicator aequorin has shown discrete spots with < 1 \(\mu\)m diameter at the squid giant synapse, and the duration of the luminescence signal was brief (~1 ms; [13]). Measurements with a moveable confocal spot-detection system and a low-affinity Ca\(^{2+}\)-indicator at the cultured neuromuscular junction showed ‘hot-spots’ of Ca\(^{2+}\)-entry and fast decay (~2 ms) of [Ca\(^{2+}\)] within hot spots [14]. More recently, near-membrane [Ca\(^{2+}\)] signals were measured with total internal reflection fluorescence microscopy (TIRFM) techniques in bipolar neurons and inner hair cells [15], and in chromaffin cells [16]. These studies showed local ‘hot spots’ of Ca\(^{2+}\) elevations, probably corresponding to sites of vesicle fusion. The question of how high local [Ca\(^{2+}\)] must rise, and how fast it must decay to evoke transmitter release with the same efficiency and time-course as during a presynaptic AP, however, remained unclear until a few years ago.
The reverse approach

When interpreting data obtained from microscopic imaging, it must be considered that optical imaging is limited to a spatial resolution in the range of the wavelength of light. The local [Ca\textsuperscript{2+}] signal, however, has gradients in the order of 10–100 nm, shorter than this limit. Furthermore, direct imaging of Ca\textsuperscript{2+} with indicator dyes produces a signal that is low-pass filtered by the Ca\textsuperscript{2+}-binding and unbinding kinetics of the dye. This means that the signal of interest (free [Ca\textsuperscript{2+}]) can only be obtained by an extrapolation — both in time and space — of the measured signal, taking into account the kinetics of the indicator dye, and the point spread function of the microscope objective.

An alternative approach to gain insight into the Ca\textsuperscript{2+} requirements of vesicle fusion uses Ca\textsuperscript{2+} uncaging to stimulate vesicle fusion. It makes use of the expectation that Ca\textsuperscript{2+} uncaging produces spatially homogenous [Ca\textsuperscript{2+}] elevations in small cells [17]. The spatial homogeneity ensures that the fluorescent Ca\textsuperscript{2+} indicator and the vesicle fusion machinery ‘see’ the same [Ca\textsuperscript{2+}] elevation. Therefore, the [Ca\textsuperscript{2+}]; elevation that elicits secretion can be measured. The amount and kinetics of release are then related to the [Ca\textsuperscript{2+}], and kinetic modeling is used to fit the data. Finally, knowledge of the parameters of a minimal kinetic model for Ca\textsuperscript{2+}-activated vesicle fusion enables the experimenter to infer the local [Ca\textsuperscript{2+}], signal compatible with the transmitter release time-course during a physiological stimulus. This approach can be seen as a ‘reverse’ approach of assessing the local (microdomain) [Ca\textsuperscript{2+}], for transmitter release [18].

The Ca\textsuperscript{2+} uncaging approach has been applied in secretory cells, such as neuroendocrine cells [19,20]. In the nervous system, Ca\textsuperscript{2+} uncaging has been applied in preparations with accessible presynaptic structures, especially cells with ribbon-type synapses, such as bipolar neurons in the retina [21], inner hair cells in the cochlea [22] and recently photoreceptor cells [23,24]. Ca\textsuperscript{2+} uncaging has also been applied to a large CNS synaptic terminal, the calyx of Held, which has emerged as a powerful model system to study presynaptic Ca\textsuperscript{2+} signaling and transmitter release. The calyx of Held is a large glutamatergic synapse located in the auditory brainstem pathway. Electron microscopic studies have estimated that each calyx contains ~500 active zones [4,25], which is in agreement with a functional estimate of the number of active zones based on excitatory postsynaptic current (EPSC) fluctuation analysis [26]. The calyx of Held is large (~10–15 μm), enabling direct whole-cell recordings of this synaptic terminal [27,28]. This, in turn, enables the experimenter to perform Ca\textsuperscript{2+} uncaging while the terminal is under voltage- or current clamp.

Ca\textsuperscript{2+} uncaging at the calyx of Held has shown a surprisingly high Ca\textsuperscript{2+}-sensitivity of vesicle fusion. Step-like [Ca\textsuperscript{2+}], elevations by Ca\textsuperscript{2+} uncaging evoked notable transmitter release at 1–2 μM [29,30], and with [Ca\textsuperscript{2+}], steps of > 10 μM a pool of readily releasable vesicles was rapidly depleted [30,31*]. Transmitter release evoked by Ca\textsuperscript{2+}-uncaging leading to [Ca\textsuperscript{2+}], steps of > 10 μM inhibited release induced by subsequent presynaptic depolarizations [30] and vice versa [31*], suggesting that the same pool of readily releasable vesicles was released by both types of stimuli. Transmitter release rates showed a steep, highly non-linear dependence on [Ca\textsuperscript{2+}], with a slope of 4–4.5 in the double-logarithmic plot of release rate as a function of [Ca\textsuperscript{2+}], in the range of 2–8 μM (see Figure 1a; [30,29]). The slope of ~4 confirms the classical finding of high cooperativity of Ca\textsuperscript{2+} in triggering transmitter release [32] that is obtained by varying the extracellular Ca\textsuperscript{2+} concentration. The Ca\textsuperscript{2+} uncaging work shows, in keeping with earlier studies in neuroendocrine cells [19,20], bipolar cells [21] and crayfish motor neuron terminals [33], that the high Ca\textsuperscript{2+}-cooperativity (~4–5) arises from the non-linear action of Ca\textsuperscript{2+} at the Ca\textsuperscript{2+} sensor for vesicle fusion, as opposed to possible non-linearities interposed between variations of extracellular [Ca\textsuperscript{2+}] and the intracellular [Ca\textsuperscript{2+}], reached at the sites of vesicle fusion.

The Ca\textsuperscript{2+}-dependency of release rates and synaptic delays obtained from Ca\textsuperscript{2+}-uncaging experiments (Figure 1a) were fitted with minimal kinetic models, which assumed that five Ca\textsuperscript{2+} ions have to bind before vesicle fusion occurs (Figure 1b). Both studies at the calyx of Held [29,30] found that five Ca\textsuperscript{2+} ions were needed to accommodate the large slope (>4) in the double-logarithmic plot of release rate versus [Ca\textsuperscript{2+}], (Figure 1a), but the two proposed models differed in the mechanism of Ca\textsuperscript{2+}-binding, which was assumed either to be independent [29] or to have an intrinsic cooperativity [30]. With the parameters of a minimal kinetic model of Ca\textsuperscript{2+}-binding and vesicle fusion fixed by fitting the Ca\textsuperscript{2+} uncaging data (Figure 1a), the waveform of the local [Ca\textsuperscript{2+}], signal was then inferred as that particular waveform which, when driving the kinetic models, reproduced the time-course of release during a presynaptic AP. The inferred local [Ca\textsuperscript{2+}], waveforms had brief durations (half-width, 0.4–0.5 ms), barely longer than the Ca\textsuperscript{2+}-current measured during an AP at the calyx of Held [34,35]. The two models yielded peak [Ca\textsuperscript{2+}], amplitudes of ~10 μM [29] and ~25 μM [30] (Figure 1c). These estimates were significantly lower than the range of 75–300 μM inferred previously by theoretical studies [11,12], by somewhat more indirect amplitude estimates at invertebrate synapses [36,13], and by Ca\textsuperscript{2+}-uncaging experiments in bipolar cells [21].

What can we learn from Ca\textsuperscript{2+} uncaging about the mechanisms that determine the timing of transmitter release during the physiological stimulus? Figure 2a shows simulations made with a model of Ca\textsuperscript{2+}-binding and vesicle fusion [30] for a series of brief, local [Ca\textsuperscript{2+}], signals. The
local \([\text{Ca}^{2+}]_i\) signals had the same time course as inferred from the reverse approach (Figure 1c), but were scaled to different amplitudes in the range of 5–25 \(\mu\)M. A series of such \([\text{Ca}^{2+}]_i\) signals predicts a 700-fold range of peak transmitter release rates (Figure 2a,b), but the timing between the local \([\text{Ca}^{2+}]_i\) signal and the peak transmitter release is changed only minimally, by \(\Delta t \approx 50\) ms. This illustrates a well-known property of AP-evoked transmitter release, namely the stability of the timing of transmitter release against changes in the amount of transmitter release [37,38]. A small change in time difference between the peak presynaptic \(\text{Ca}^{2+}\)-current and the peak transmitter release rate, compatible with the prediction of the model (see Figure 2b), has been found.

Steps involved in the ‘reverse’ approach of assessing the local \([\text{Ca}^{2+}]_i\) signal for transmitter release. (a) Measurement. Rate of transmitter release (left panel) and synaptic delays (right panel) as a function of the \([\text{Ca}^{2+}]_i\), measured in \(\text{Ca}^{2+}\) uncaging experiments at the calyx of Held. Note the double-logarithmic scales in the left panel, and the steeply non-linear relation in the range of 2–8 \(\mu\)M \([\text{Ca}^{2+}]_i\) (slope, 4.2 in double-logarithmic coordinates). (b) Model. This model assumes that five \(\text{Ca}^{2+}\) ions bind in a cooperative fashion (\(b < 1\)) before vesicle fusion occurs with a rate constant \(g\). The model predicted the \(\text{Ca}^{2+}\)-dependency of release rates and the synaptic delays after \(\text{Ca}^{2+}\)-uncaging (continuous lines in [a]). For model parameters, see Schneggenburger and Neher [30]. (c) Backcalculation. Black traces show measured EPSCs in response to presynaptic APs (upper panel) and the corresponding average release rate obtained by deconvolution of the measured average EPSC with the mEPSC waveform (lower panel, dashed trace). When the release model in (b) is driven with the inferred local \([\text{Ca}^{2+}]_i\) signal (red trace, lower panel), a transmitter release rate (black continuous trace, lower panel) similar to the observed one is obtained. The dashed red line is a Gaussian with half-width of 0.36 ms, a waveform similar to the presynaptic \(\text{Ca}^{2+}\)-current during an AP [35]. Reproduced, with permission, from Schneggenburger and Neher [30].
recently at the calyx of Held (\sim 100 \mu s; [39*]). Thus, simple models with multiple Ca^{2+}-binding steps followed by vesicle fusion [21,29,30] can explain the rapid time course of transmitter release, as well as the stability of the release time-course upon varying the transmitter release probability.

The reverse approach for estimating the local [Ca^{2+}]_i assumes that the transmitter release kinetics are determined entirely by the rise and fall of the local [Ca^{2+}]_i signal. This assumption has recently been validated more directly by experiments at the calyx of Held [40*]. In this study, transient [Ca^{2+}]_i elevations in the milliseconds range were evoked by UV-laser induced Ca^{2+}-uncaging. The amplitude and the rise time of EPSCs increased with the half-width of the [Ca^{2+}]_i transients. Importantly, EPSCs with a rise-time as short as that of AP-evoked EPSCs were only obtained when the measured [Ca^{2+}]_i transients were shorter than 0.5 ms, confirming the width of the local [Ca^{2+}]_i signal estimated by the reverse approach [29,30]. In addition, these experiments [40*] show directly that the rise and fall of the local [Ca^{2+}]_i near the Ca^{2+}-sensors for vesicle fusion determines the amount and the kinetics of transmitter release.

The local arrangement of Ca^{2+} channels at the active zone

What are the implications of the new estimates of the local [Ca^{2+}]_i signal for our understanding of the arrangement of readily releasable vesicles relative to Ca^{2+} channels? Two salient features of the estimated local [Ca^{2+}]_i at the calyx of Held are the brief duration of this signal (\sim 0.5 ms), and its relatively low amplitude (\sim 10–20 \mu M; see above). If only a single Ca^{2+} channel controlled the release of a given vesicle, then the Ca^{2+}-channel–vesicle distance could be calculated, given that the single-channel current and the properties of the intracellular Ca^{2+}-buffers are known [41]. The single-channel conductance of presynaptic Ca^{2+} channels (mainly P/Q-type channels, but also N- and R-type channels; see [42] and references therein) are, however, not known, and the properties, concentration and identity of Ca^{2+}-buffers are also unknown for most presynaptic terminals. If only ATP and a non-mobile (fixed) endogenous Ca^{2+}-buffer were present, a local [Ca^{2+}]_i transient with peak amplitude of 10 \mu M would be produced by a single Ca^{2+}-channel with 0.2 pA of current flow, located at a distance of 30–40 nm from a readily-releasable vesicle [43]. Is such a simplifying assumption justified, however?

There is an ongoing debate on whether release is controlled by a single Ca^{2+}-channel in the immediate vicinity of the vesicle (‘single channel domain’), or by multiple channels (‘domain overlap’). In the absence of direct ultrastructural information, the only answer to this question comes from the apparent power relationship between transmitter release and Ca^{2+}-current in experiments in which the number of open Ca^{2+}-channels is varied during AP-like stimuli [43–45]. If a single Ca^{2+}-channel dominates the local [Ca^{2+}]_i signal of a given vesicle (single
The spacing between a vesicle and its release-controlling Ca\(^{2+}\)-channel(s) has also been assessed by studying the effects of intracellular application of the synthetic Ca\(^{2+}\)-chelators, EGTA or BAPTA into the presynaptic terminal. Both Ca\(^{2+}\)-chelators have similar equilibrium binding for Ca\(^{2+}\), but the on-rate of Ca\(^{2+}\)-binding to BAPTA is \(\sim\)100 times faster than the one of EGTA. Thus, BAPTA chelates Ca\(^{2+}\) very briefly after entering a cell, whereas EGTA Ca\(^{2+}\) can diffuse further away from the entry site before being bound. At the squid giant synapse even high (up to 80 mM) concentrations of intracellular EGTA left transmitter release unaffected, whereas BAPTA suppressed release dose-dependently in the low millimolar range [36]. Conversely, at the calyx of Held [34] and at several other CNS synapses [46,47], EGTA suppressed release by about 50% at concentrations of 1–10 mM. The experiments with BAPTA and EGTA and the apparent power relationship upon varying the number of open Ca\(^{2+}\)-channels (see above) suggest, therefore, that release at the squid giant synapse is controlled by a tight co-localization between a vesicle and a single Ca\(^{2+}\)-channel, with a distance so close (near-molecular coupling) that the slow buffer EGTA becomes completely ineffective. However, at the calyx of Held and at other mammalian excitatory CNS synapses, the Ca\(^{2+}\)-channel-vesicle co-localization is not quite as tight, with several Ca\(^{2+}\)-channels probably influencing the release of a given vesicle [34,43]. The answer to the question of whether a single Ca\(^{2+}\)-channel is able to trigger release also depends on the single channel flux, and, therefore, on the extracellular [Ca\(^{2+}\)]. Differences in the extracellular [Ca\(^{2+}\)] have recently been proposed to explain some of the differences reported among synapses [45].

A recent modeling study [43] of presynaptic Ca\(^{2+}\)-diffusion and release-activation takes into account most of the functional and ultrastructural data obtained at the calyx of Held. The authors concluded that the average Ca\(^{2+}\)-channel-vesicle distance must be large (>200 nm) [43], and that a variable Ca\(^{2+}\)-channel-vesicle distance across active zones could adequately explain the relative efficiencies of EGTA and BAPTA in reducing release probability at the calyx of Held. They also showed that more than 10 Ca\(^{2+}\)-channels are needed for the release control of a given vesicle to account for the high Ca\(^{2+}\)-cooperativity (\(\sim\)3–4) upon modulating the number of open Ca\(^{2+}\)-channels. Finally, the large average distance (>200 nm), the requirement for more than 10 channels, and the known active zone diameter (\(\sim\)250 nm [4]) led Meinrenken et al. [43] to propose a vesicle-Ca\(^{2+}\)-channel
topography in which a tightly packed cluster of channels, acting somewhat like a single Ca\textsuperscript{2+} entry point, controls the release of readily-releasable vesicles positioned at random distances over the active zone (Figure 3c). This is an interesting proposal, synthesizing a large amount of available data. However, alternative geometrical arrangements need to be explored in further simulations after some critical parameters, such as the single Ca\textsuperscript{2+}-channel conductance, have been constrained by experimental results. Ultrastructural analysis might also provide crucial data. Recently, a highly sensitive freeze-fracture labeling technique has revealed the localization of postsynaptic glutamate receptors of the AMPA-subtype at single synaptic sites [48]. It would be useful in the future to study the location of single Ca\textsuperscript{2+}-channels within the presynaptic active zone with a similar spatial resolution.

Physiological implications of a high Ca\textsuperscript{2+} cooperativity of release

What might be the advantage of the high cooperativity of Ca\textsuperscript{2+} in inducing vesicle fusion at synapses? The answer could lie in a maximized signal to background ‘noise’ ratio for synaptic transmission. During a presynaptic AP, the peak transmitter release rate is 10\textsuperscript{6}–10\textsuperscript{7} fold higher than the spontaneous rate of ‘miniature’ postsynaptic currents; a relative increase that is much larger than the relative increase in presynaptic [Ca\textsuperscript{2+}], by which it is driven (from a basal value of ~50 nM, to 10–20 \mu M local [Ca\textsuperscript{2+}]; see above). This can only be achieved with a highly nonlinear transduction mechanism. Thus, the nonlinear, cooperative action of Ca\textsuperscript{2+} at the molecular machinery [5–8] that triggers vesicle fusion might have evolved to enable a high (~10\textsuperscript{3}–10\textsuperscript{6} fold) dynamic range of peak transmitter release rate as compared to that of unstimulated background release rate.

The high Ca\textsuperscript{2+}-cooperativity (~4–5; see above) is also crucial for explaining short-term plasticity of transmitter release, such as synaptic facilitation, as was first pointed out by Katz and Miledi [49]. Recently, it was estimated that about one-third of the facilitation induced by elevations in residual Ca\textsuperscript{2+} can be explained if it is assumed that residual Ca\textsuperscript{2+} adds linearly to the local [Ca\textsuperscript{2+}], signal, and thereby increases the local [Ca\textsuperscript{2+}] signal for transmitter release [50\textsuperscript{*}]. The remaining facilitation was mediated neither by an increased Ca\textsuperscript{2+}-sensitivity as assayed by Ca\textsuperscript{2+}-uncaging nor by an increased readily releasable vesicle pool size. To explain the discrepancy, a small supralinearity in the summation of Ca\textsuperscript{2+}-signals was postulated, possibly caused by partial saturation of an endogenous Ca\textsuperscript{2+}-buffer [41]. A role of Ca\textsuperscript{2+}-buffer saturation in facilitation was also found at the mossy-fiber–CA3 synapse, where the Ca\textsuperscript{2+}-binding protein calbindin is expressed presynaptically [51\textsuperscript{*}].

The high Ca\textsuperscript{2+}-cooperativity in inducing vesicle fusion will also make small changes in the number of open Ca\textsuperscript{2+}-

channels highly effective in modulating transmitter release, at least when multiple Ca\textsuperscript{2+}-channels control the release of a given vesicle (domain overlap). A modulation of the number of open Ca\textsuperscript{2+}-channels occurs during paired-pulse stimulation, which leads to Ca\textsuperscript{2+}-
current facilitation in the presynaptic terminal [52,53], a mechanism specific for the presynaptic P/Q-type Ca\textsuperscript{2+}-channels [53,54\textsuperscript{*}]. Ca\textsuperscript{2+}-current facilitation is expected to contribute to paired-pulse facilitation of transmitter release, but its exact contribution is yet to be determined. Modulation of the number of open Ca\textsuperscript{2+}-channels also occurs after activation of presynaptic, G-protein coupled receptors, many of which lead to a direct, \beta\gamma-mediated downregulation of presynaptic Ca\textsuperscript{2+}-current (see Kimura \textit{et al.} [55] and references therein).

Conclusions

Recent work, mostly conducted at the calyx of Held, has emphasized the role of the local [Ca\textsuperscript{2+}]; signal in vesicle fusion. Ca\textsuperscript{2+}-uncaging has shown that the intracellular Ca\textsuperscript{2+}-sensitivity for vesicle fusion is higher than classically assumed, with strong rates of release obtained at ~10 \mu M [Ca\textsuperscript{2+}]; [29,30]. Ca\textsuperscript{2+} acts in a highly cooperative fashion at the Ca\textsuperscript{2+}-sensor for vesicle fusion, and the Ca\textsuperscript{2+}-sensors are not saturated during the local [Ca\textsuperscript{2+}] signal induced by a presynaptic AP. This, together with the finding that release is controlled by multiple Ca\textsuperscript{2+}-channels in a domain overlap situation [43], means that small changes in Ca\textsuperscript{2+} influx during a presynaptic AP will be highly effective in modulating the transmitter output at synapses. In addition, Ca\textsuperscript{2+}-binding to the Ca\textsuperscript{2+}-sensor for vesicle fusion is not at equilibrium during the short duration of the local [Ca\textsuperscript{2+}] signal and, thus, prolonging the decay of the local [Ca\textsuperscript{2+}], will lead to an increased release probability. Thus, the local [Ca\textsuperscript{2+}] signal reached at the sites of vesicle fusion determines the transmitter release probability during short-term modulation of transmitter release, beside the availability of readily releasable vesicles. Important questions to be asked in the near future, are: first, is the intracellular Ca\textsuperscript{2+}-sensitivity of vesicle fusion or the Ca\textsuperscript{2+}-channel–vesicle distance modulated during developmental maturation, or during longer-lasting modulation of transmitter release? Second, are there separate Ca\textsuperscript{2+}-sensors, possibly located on separate pools of readily releasable vesicles [6], for the phasic and for the asynchronous components of transmitter release? Finally, a closer look into the molecular mechanisms mediating the extremely non-linear action of Ca\textsuperscript{2+} in triggering vesicle fusion would be very useful for further research in this area.

Update

Three studies of immediate interest for presynaptic Ca\textsuperscript{2+} control of vesicle fusion have been published recently. First, Millar \textit{et al.} [56] have compared the intracellular Ca\textsuperscript{2+} sensitivity of two types of crayfish neuromuscular synapses that have widely different short-term plasticity: phasic
(depressing) synapses, and tonic (facilitating) synapses. They showed that presynaptic \([Ca^{2+}]\) steps with similar amplitudes, produced by \(Ca^{2+}\) uncaging, evoked the delayed release of only few quanta in tonic synapses, but stronger release with shorter delay in phasic synapses, despite similar numbers of readily-releasable pool vesicles in both types of nerve terminals. This indicates that depressing and facilitating synapses have a differential intracellular \(Ca^{2+}\)-sensitivity for vesicle fusion, and that the release machinery of depressing synapses uses \(Ca^{2+}\) more efficiently. In addition, the authors propose that \(Ca^{2+}\)-dependent vesicle priming mediates the strong facilitation observed in tonic synapses.

Second, Lou et al. [57] have studied the potentiation of transmitter release by phorbol esters, which target pre-synaptic protein-kinase C and/or munc-13 pathways. By using \(Ca^{2+}\) uncaging at the calyx of Held, they showed that phorbol esters potentiate release by increasing the intracellular \(Ca^{2+}\)-sensitivity of vesicle fusion, concomitant with a decrease in the apparent \(Ca^{2+}\)-cooperativity. They also showed that the high \(Ca^{2+}\) cooperativity in inducing vesicle fusion in the range of 2–8 \(\mu M\) (≈ 4–5) is gradually reduced at lower \([Ca^{2+}]\), reaching a value of ≈ 1 at baseline \([Ca^{2+}]\). They explain their data with a novel ‘allosteric’ model of \(Ca^{2+}\)-triggering of vesicle fusion, in which release can occur both from fully occupied \(Ca^{2+}\)-sensors and, at low rates, from partially bound or unbound \(Ca^{2+}\)-sensors. This work offers a framework for understanding the modulation of the \(Ca^{2+}\)-sensitivity of transmitter release during long-lasting forms of presynaptic plasticity, and it sheds new light on the relation between phasic release (driven by brief local \([Ca^{2+}]\) transients) and asynchronous release, which both might be mediated by the same \(Ca^{2+}\)-sensor for vesicle fusion.

Finally, Fedchyshyn and Wang [58] showed a developmental transformation of \(Ca^{2+}\)-channel–vesicle coupling at the calyx of Held. In agreement with previous work ([34], and see above), intracellular application of low millimolar concentrations of EGTA suppressed transmitter release in young calyces of Held, and the \(Ca^{2+}\)-cooperativity upon varying the number of open \(Ca^{2+}\)-channels during presynaptic voltage-clamp was high (≈ 4–5), indicating that several \(Ca^{2+}\)-channels controlled the release of a given readily-releasable vesicle. In older animals, however, the sensitivity to intracellular application of EGTA and the \(Ca^{2+}\)-cooperativity upon changing the number of open \(Ca^{2+}\)-channels were reduced. Both observations indicate that with developmental maturation, the spatial coupling between \(Ca^{2+}\)-channels and readily-releasable vesicles becomes tighter, with fewer \(Ca^{2+}\)-channels controlling the release of a given vesicle.

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- of outstanding interest


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The authors used membrane capacitance measurements to study the kinetics of vesicle fusion in photoreceptor cells. It was found that 100 ms depolarizations in a membrane potential range of –60 to –10 mV led to capacitance increases that were linearly related to the Ca$^{2+}$-current. Also, with Ca$^{2+}$-uncaging between 0.5–3 μM Ca$^{2+}$, the rate of secretion was related nearly linearly to [Ca$^{2+}$], but higher [Ca$^{2+}$], were not investigated. The authors suggest that photoreceptor cells possess a highly Ca$^{2+}$-sensitive pool of vesicles, with an intrinsic Ca$^{2+}$-cooperativity lower than that found in other neurons.


The authors performed presynaptic capacitance measurements and presynaptic Ca$^{2+}$-uncaging to determine the Ca$^{2+}$ sensitivity of vesicle fusion at the calyx of Held. With step-like Ca$^{2+}$ elevations > 10 μM, a pool of readily releasable vesicles was depleted. Rates of pool depletion were 2–3 ms at 10 μM Ca$^{2+}$, and < 1 ms for Ca$^{2+}$ steps > 30 μM, concurring with the previously estimated Ca$^{2+}$-sensitivity at the calyx of Held [29,30], which relied on EPSCs as a measure of release. Sub-millisecond kinetic properties of vesicle fusion were reached at significantly lower Ca$^{2+}$ than at ribbon-type synapses in bipolar nerve terminals [21] and inner hair cells [22].


The authors observed that strong presynaptic membrane depolarizations made in the presence of Ca$^{2+}$-channel blockers neither influenced the amount nor the kinetics of transmitter release evoked by Ca$^{2+}$-uncaging at the calyx of Held. This shows that membrane potential, besides its well-known effect on Ca$^{2+}$-channel open probability, has no direct effect on transmitter release rates, as opposed to what is postulated in the ‘Ca$^{2+}$-voltage-hypothesis’ [38]. The authors also demonstrated a small decrease in the latency between presynaptic Ca$^{2+}$-tail currents and the peak of phasic transmitter release, as predicted by a simple model of Ca$^{2+}$-control of vesicle fusion [39]. However, the absolute peak time difference was slightly larger in the experiments than predicted by the model (by ~200 μs; see also [29]).


The authors investigated the presynaptic, Ca$^{2+}$-dependent mechanism of short-term facilitation with paired pre- and postsynaptic voltage-clamp recordings at the calyx of Held, and with presynaptic Ca$^{2+}$-uncaging. They show that the Ca$^{2+}$ sensitivity of transmitter release was unchanged.
during facilitation, and concluded that an increased local $[\text{Ca}^{2+}]_i$ signal mediates facilitation. A small supra-linearity in the summation of residual and local $\text{Ca}^{2+}$-signal that might be caused by (partial) saturation of an endogenous $\text{Ca}^{2+}$-buffer [41,51] was postulated to fully explain facilitation.


This study presents evidence that saturation of the $\text{Ca}^{2+}$-buffer calbindin-D28k in presynaptic terminals contributes to paired-pulse facilitation of transmitter release. IPSPs recorded in cortical pyramidal cells increased during whole-cell recording of the presynaptic neuron (multipolar bursting cells that express calbindin-D28k), and adding calbindin-D28k to the pipette solution prevented the increase of IPSP amplitude. These observations indicate that in these synapses, calbindin-D28k normally reduces the release probability by buffering incoming $\text{Ca}^{2+}$, but that (partial) saturation of calbindin-D28k during a first AP can lead to a supra-linear increase of the local $[\text{Ca}^{2+}]_i$ that triggers release in response to a second stimulus (see also [50]). Further evidence for a role of calbindin-D28k as a saturable $\text{Ca}^{2+}$-buffer in facilitation was obtained at the mossy-fiber–CA3 cell synapse.


The authors studied presynaptic $\text{Ca}^{2+}$-currents and synaptic transmission at the calyx of Held synapse in mice lacking the pore-forming $\alpha_{1A}$ subunit of the P/Q-type channel. EPSCs were reduced only slightly in the $\alpha_{1A}$ knockout mice, showing that presynaptic N-type $\text{Ca}^{2+}$-channels compensated for the loss of the P/Q-type channel. However, paired-pulse facilitation of EPSCs was reduced, and the $\text{Ca}^{2+}$-dependent facilitation of presynaptic $\text{Ca}^{2+}$-currents [52,53] was absent in the knockout mice. This shows that $\text{Ca}^{2+}$-current facilitation is a specific property of presynaptic P/Q-type $\text{Ca}^{2+}$-channels.


