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Neurotransmitter Release: Synchronous and Asynchronous

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Introduction

More than 50 years ago, Fatt and Katz showed that an action potential (AP) arriving at the frog neuromuscular junction releases the chemical neurotransmitter ACh in multimolecular packages which they called quanta. By that time, the quantum was detected on the basis of the conductance change induced in the follower cell – that is, the postsynaptic response which either occurred as an AP evoked response or as miniature postsynaptic activity (‘mini’). Minis were recorded when AP generation was prevented by blocking voltage-activated sodium channels with tetrodotoxin (TTX). Fluctuating evoked responses were observed when lowering the ratio of extracellular Ca\(^{2+}\)/Mg\(^{2+}\) concentration. In this way, the AP-induced multiquantal end plate potential could gradually be decomposed to postsynaptic potentials containing increasingly fewer quanta, down to one quantum which equaled the mini.

Based on these observations, del Castillo and Katz assumed that the spontaneous asynchronous mini activity is the product of the same release machinery as the quantum in the AP-induced evoked postsynaptic response. In other words, evoked transmitter release was attributed to the synchronous occurrence of the same quanta that constitute mini activity. Thus, the evoked release would merely reflect a massive acceleration of spontaneous release. Whether or not this assumption is correct is the main topic of this article.

Definitions

In the central nervous system, one presynaptic nerve cell typically forms hundreds or thousands of synapses with multiple follower cells. The structural definition of a synapse commonly refers to one specialized contact comprising, on the presynaptic side, between one (some hippocampal inhibitory neurons) and hundreds (calyx of Held) of active zones and, on the postsynaptic side, a corresponding number of postsynaptic specializations that contain the transmitter receptors at high density. The set of synapses formed between two neurons is commonly referred to as a synaptic connection. The number of vesicles released at one synaptic connection varies, similar to the neuromuscular junction, in a quantal manner, with the variance of the release process being smaller the larger the number of active zones contained in the connection between two cells. The total number of quanta released by one presynaptic cell to one follower cell is called the quantum content of a synaptic response, and the total amount of transmitter released is given by the product of the quantum content and the number of transmitter molecules per quantum. Thus, the quantum content can be viewed as the presynaptic indicator of synaptic strength. When it is not possible to record unitary postsynaptic responses (i.e., the response to activation of just one synaptic connection), the frequency of minis is often taken as an indirect estimate of synaptic strength. This again raises the question to what extent observations made with minis are attributable to mechanisms of evoked release.

However, before going into greater detail, it is necessary to agree on a stricter use of the respective terminology because terms such as ‘asynchronous release,’ ‘mini activity,’ ‘tonic release,’ and ‘spontaneous release’ are often used interchangeably. Furthermore, it is not always clear whether the terms ‘asynchronous’ or ‘spontaneous’ refer to what is to be regarded as constitutively active quantal release (i.e., a form of release that can be distinguished by being Ca\(^{2+}\) independent).

Because Ca\(^{2+}\) concentration in presynaptic terminals and Ca\(^{2+}\) sensitivity of the exocytotic machinery are the two most critical determinants of the speed of fusion, Ca\(^{2+}\) sensitivity of postsynaptic activity may serve to distinguish between four different modes of the underlying release process: the highly synchronous AP-evoked response (with a \(K_D\) of \(\sim 10\) \(\mu\)M; data from calyx of Held); the asynchronous delayed response, as induced by high-frequency AP generation (or prolonged presynaptic depolarization) (\(K_D\) \(\sim 1.5\) \(\mu\)M; data from GABAergic synapses in culture); mini activity (\(K_D\) \(< 1\) \(\mu\)M); and constitutively active release (no Ca sensitivity). The release underlying the first three modes can, in a broad sense, be referred to as ‘regulated,’ whereas the constitutively active release is ‘unregulated.’

The capacity for constitutively active release in the form of quanta might be a property of many, if not all, cells and is to be expected if the cytosolic concentration of a molecule reaches a critical level. For instance, by using appropriate cellular sensors, Poo and colleagues demonstrated that muscle cells loaded with the neurotransmitter acetylcholine soon start secreting this foreign transmitter in a pulsatile manner reminiscent of minis.
Minis are still to be defined as synaptic responses to transmitter release in the absence of AP generation. As in the original experiments on the frog neuromuscular junction, they are recorded in the presence of TTX. The term 'spontaneous synaptic activity' is commonly reserved for postsynaptic events recorded in the absence of TTX. This activity may therefore reflect all four forms of release.

An interesting question is whether or not minis always reflect a completely stochastic release process. By applying nonlinear methods to quantify any detected determinism in spontaneous release, Walmsley and colleagues presented evidence for the nonrandom nature of spontaneous inhibitory postsynaptic currents in mouse auditory neurons, which suggests that minis might not be very 'spontaneous' in their origin.

In any case, minis should not be equaled with basal constitutively active transmitter release, because in most neurons their frequency displays clear sensitivity to changes in extra- and intracellular Ca$^{2+}$ concentration and to activation of presynaptic membrane receptors with access to intracellular Ca$^{2+}$ signaling pathways at 'resting' conditions. However, mini activity recorded from single synapses under physiological Ca$^{2+}$ concentration may include a fraction of exocytotic events that are Ca$^{2+}$ independent and therefore fall into the category of constitutively active secretion. To isolate this type of mini activity, it might be necessary to inactivate some of the molecular elements that provide Ca$^{2+}$ sensitivity to the fusion process. Also, as an approximation, one may acquire the minis which persist in Ca$^{2+}$-free extracellular salt solutions. In cortical cultures, only a very small percentage of synapses (~1% according to Murphy and colleagues) are able to sustain spontaneous release in the absence of Ca$^{2+}$. The very scarce Ca$^{2+}$-independent spontaneous activity can, however, be enhanced by manipulations such as application of hyperosmolar solutions or z-latrotoxin.

**Possible Function of Asynchronous Mini Activity**

The prevailing viewpoint is that synchronous evoked and asynchronous mini activity serve distinct functions and have a different origin. It is obvious that the synchronous evoked release is the major means to transmit a precise temporal pattern of presynaptic activity to the follower cell. Therefore, the underlying fusion activity is the most tightly controlled form of release. This tight control generally results in a rather low probability of vesicle release (~0.1–0.3), so that upregulation and also some downregulation can occur. In the course of synapse development, this form of release is achieved with some delay after an initial period during which release is more asynchronous.

Concerning minis, data are accumulating in favor of the idea that this form of asynchronous synaptic activity has its specific function in the maintenance of synapses. The group of Gähwiler and Thompson compared the effect of TTX and clostridial toxins on developing hippocampal neurons in slice cultures from the rodent brain. Because TTX prevented evoked release, whereas treatment with BoNT prohibited any form of release, they were able to examine the relative impact of synchronous evoked versus asynchronous mini release on synapse formation and maintenance. They concluded that maintenance of dendritic spines critically depends on the generation of mini activity, whereas evoked release is not required. Other studies supported the idea that mini activity may have a 'trophic' function, even though in intact brain preparations the mini frequency is typically rather low (typically 1–5 s$^{-1}$). Considering that one hippocampal neuron carries approximately 50 000 synapses, one has to conclude that only one quantum every 3 h can keep a synapse in place. On the other hand, it might suffice that a dendritic branch or an entire dendrite 'sees' an active presynaptic terminal. Schuman and colleagues showed that NMDA receptor-mediated minis stabilize synaptic function through active suppression of dendritic protein synthesis.

The notion that mini activity represents a specific form of synaptic communication is further supported by the observation that appropriate activation of presynaptic nicotinic acetylcholine receptors, metabotropic glutamate receptors, or TrkB receptors (the receptors for the brain-derived neurotrophic factor), among others, can influence the activity of the follower neuron. Of course, the conductance change associated with the release of a single vesicle is quite small (105–180 pS for the AMPA receptor response to a glutamate-containing vesicle and 105–135 pS for a GABA-containing vesicle), but there are synaptic connections in the central nervous system that solely operate by releasing at the most one quantum per AP. Also, experiments have shown that this may be enough to modify the activity of the target cell, especially if the latter has a high input resistance. Regehr and colleagues showed that in cerebellar stellate cells single inhibitory quanta suppressed firing, whereas coincident excitatory quanta reliably and rapidly triggered firing. However, even in the relatively low resistance neurons of the cerebral cortex, nicotinic input (smoking) was shown to influence mini activity to a degree that cortical networks could be affected.
Together, these data justify a closer examination of the origin of minis as opposed to evoked transmitter release.

**Different Vesicle Pools for Synchronous and Asynchronous Release?**

Since the times of Katz, enormous effort has been focused on the characterization of distinct vesicle pools since it has become obvious that the individual vesicles vary with regard to their distance to the active zones, the site of presynaptic Ca\(^{2+}\) channel clusters, the release kinetics of directly monitored individual fusion events, and the incidence of labeling of endocytosed vesicles with styryl dyes. Most important, some vesicles are more readily released than others. The question arose of whether or not minis are preferentially generated by a distinct, more 'reluctant' vesicle pool. This pool would be replenished at rest and release vesicles more slowly than the readily releasable pool of vesicles. However, when vesicles were labeled with different styryl dyes during rest and activity, it became clear that there is a significant amount of cross-depletion of both vesicle pools, which supports the idea that synchronous AP-mediated and asynchronous mini release draw upon the same vesicle pool.

**Differential Molecular Control of Synchronous versus Asynchronous Release?**

In 1971, Quastel et al. suggested the existence of a final step in exocytosis that is not dependent on Ca\(^{2+}\). Today, the experimental means exist to uncover the basal constitutively active spontaneous release by molecular manipulations. As a rule, evoked release is prohibited by deletion of individual components of the SNARE complex, such as VAMP2, syntaxin 1, or SNAP-25. All this spares some fraction of mini activity. Typically, the remaining mini activity exhibits low Ca\(^{2+}\) sensitivity. For instance, experiments with cultured hippocampal neurons revealed that in SNAP-25 knockouts the residual spontaneous release is Ca\(^{2+}\) independent, but it can be stimulated by hypertonic solution. It is also known that the key regulator of the SNARE complex, synaptotagmin, is required for Ca\(^{2+}\)-dependent synchronous release but not for mini activity. Of particular interest are experiments by Maximov and Südhof showing that synaptotagmin is also essential for the asynchronous delayed release after a stimulus train. This clearly indicates that asynchronous release activity is associated with the synchronous evoked release (Figure 1).

**Figure 1** Synchronous and asynchronous forms of synaptic responses to activation of a single FM1-43-labeled synaptic terminal in culture. (a) Experimental scheme. One of two synaptic varicosities formed en passant by an axon crossing a dendrite is selected for direct electrical depolarization via a closely apposed patch pipette. The postsynaptic responses are recorded in the whole cell configuration from the nearby soma. (b) Phase contrast and FM1-43 fluorescence images of the respective view field (10 × 13.5 μm). (c) (Top) Presynaptic Ca\(^{2+}\) transient as acquired from the respective FM1-43 areas of the stimulated (B1) and nonstimulated (B2) boutons. In this case, Fura-2/AM was loaded for subsequent recording of the bulk Ca\(^{2+}\) signal as a measure of presynaptic activation. (Bottom) Corresponding single bouton-activated evoked inhibitory postsynaptic current (IPSC) in response to a very short (0.3 s) depolarizing pulse. (d, e) Superimposed traces of synchronous and asynchronous synaptic activity. The synchronous evoked IPSCs (eIPSCs) and asynchronous delayed IPSCs (dIPSCs), as well as the mini IPSCs (mIPSCs), were recorded in TTX. To elicit both eIPSCs and dIPSCs, the duration of the depolarizing pulse was prolonged to 3 ms.
However, all these data must not necessarily be interpreted as evidence for a totally distinct population of vesicles exclusively dedicated to asynchronous release as opposed to synchronous release. Whether a given vesicle is primed for evoked release or allowed to contribute to mini activity may depend on the actual state of the fusion switch impacting this vesicle. Results obtained by the groups of Südhof and Rothman suggest that Ca\(^{2+}\) elevation enables synaptotagmin to remove a potent fusion-suppressive action of complexin on the SNARE complex. Complexins together with synaptotagmin 1 can be regarded as a fusion clamp that arrests vesicle secretion at rest but promotes synchronous fusion upon activity. According to this model, removal of the synaptotagmin/complexin break should increase the rate of constitutive exocytosis and, vice versa, overexpression of complexins should decrease Ca\(^{2+}\)-independent mini activity. The available experimental data are in accord with this proposal. Thus, the actual intracellular Ca\(^{2+}\) concentration remains the decisive determinant of the probability of release of any given vesicle. To what extent variable endowment/access of individual vesicles with various isoforms of the SNARE protein VAMP2 or regulatory proteins such as Rab3, RIM-1, and Munc-13 determines whether or not a vesicle is prone to contribute to mini activity remains to be elucidated.

**Asynchronous Delayed Release as a Special Form of Evoked Release**

After focusing on asynchronous mini release as opposed to synchronous evoked release, we may now regard an 'intermediate' form of release, namely the asynchronous delayed liberation of presynaptic vesicles in response to prolonged or repetitive presynaptic depolarization. Under physiological test conditions, asynchronous delayed release occurs during (or soon after) a burst of presynaptic APs. It is driven by an AP-induced presynaptic Ca\(^{2+}\) elevation that usually lasts much longer than an AP. Provided that the level of this Ca\(^{2+}\) increase is still high enough to initiate exocytosis (residual Ca\(^{2+}\) hypothesis), individual vesicles will be liberated even in the absence of a preceding presynaptic AP. Thus, similar to synchronous evoked release, asynchronous delayed release requires presynaptic activity. However, in contrast to evoked release, the latencies between the arriving APs and vesicle release are (1) in general much longer than a typical synaptic delay and (2) highly variable. The latter means that asynchronous delayed events display a rather stochastic behavior (i.e., they show similarity with mini activity). Indeed, the distribution of inter-event intervals in both cases is well fitted by a single exponential function (an approximate sign of the stochastic nature of the release process). Because the frequency of spontaneous postsynaptic responses is usually quite low, asynchronous delayed instead of truly spontaneous mini responses have often been used to estimate the quantal amplitude of a given postsynaptic response.

Typically, a train of APs is activated/applied to induce asynchronous delayed release. This stimulation protocol depletes the readily releasable pool of presynaptic vesicles and induces large and long-lasting presynaptic Ca\(^{2+}\) elevations. Consequently, only newly recruited vesicles mediate asynchronous delayed release. Because vesicle recruitment is a random process, asynchronous delayed activity is composed of monoquantal responses due to the release of single vesicles. Therefore, they are often called 'miniature-like' responses. However, and despite the fact that this approach can considerably speed up the estimation of quantal size, one has to consider and, if possible, avoid stimulation-induced modifications of presynaptic as well as postsynaptic characteristics of transmission (i.e., various expressions of long-term plasticity or postsynaptic receptor desensitization).

The physiological role of asynchronous delayed release is not fully understood. The observation that asynchronous delayed release at immature synapses is usually much stronger than in their mature counterparts led to the suggestion that this form of vesicle fusion may reflect immaturity of the presynaptic release machinery. For instance, the duration of asynchronous delayed currents elicited by 50 Hz trains at GABAergic synapses decreased in the superior colliculus from 2.5 s on postnatal day 0 to 1 s on postnatal day 22. Sensory deprivation in congenitally deaf mice also resulted in much higher asynchronous delayed release than in normally developing counterparts. However, in disagreement with the 'immaturity hypothesis,' many mature synapses display asynchronous delayed release as well, even after a single presynaptic AP. For instance, cerebellar granule cell synapses on Purkinje cells or stellate cells generate after a single spike asynchronous delayed release lasting hundreds of milliseconds. Synaptic terminals in hippocampal cultures release after a single AP vesicle with a time constant of decay of 200 ms.

It should also be considered that the physiological discharge pattern of many mature neurons is not a series of solitary APs, but it often comprises bursts of APs—a condition both necessary and sufficient to initiate asynchronous delayed release. At an interneuron-to-pyramidal cell synapse of the hippocampus, a single AP produces an asynchronous delayed response with a time constant of decay of 59 ms, whereas a train of 10 APs delivered at 50 Hz prolongs...
the time constant of asynchronous delayed release to 230 ms. Auditory synapses in the chick brain stem display almost no asynchronous release at frequencies lower than 100 Hz, but at higher frequencies synaptic transmission during AP trains is mainly mediated by asynchronous release. Thus, one may conclude that, independent of the experimental preparation, high-frequency trains of 2–5 presynaptic APs have a good chance to induce an asynchronous delayed response.

Together, all these observations argue against the simple suggestion that asynchronous delayed release results from the imperfectness of release machinery. Instead, it is a complex, functionally relevant phenomenon that depends on a variety of presynaptic factors, including the availability of synaptic vesicles, Ca\(^{2+}\) sensitivity of the release machinery, and presynaptic Ca\(^{2+}\) signaling. Therefore, an individual presynaptic bouton appears not to function as a single compartment that probabilistically converts individual presynaptic APs into postsynaptic responses but instead as an integrator of many environmental and retrograde signals. This integration would affect the probability of not only evoked release but also all forms of asynchronous release. In addition, asynchronous delayed release provides a mechanism to influence follower cells for a prolonged period, after the presynaptic AP activity has subsided, and the strength of this influence would be determined both by the previous activity period and by newly arriving (retrograde or paracrine) signals.

See also: Action Potential Initiation and Conduction in Axons; Exocytosis: Ca\(^{2+}\)-Sensitivity; Fusion Pore; Ion Channel Localization in Axons; Synaptic Vesicles; Vesicle Pools.

Further Reading


