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Presynaptic origins of distinct modes of neurotransmitter release

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Abstract

Presynaptic nerve terminals release neurotransmitter synchronously, asynchronously or spontaneously. During synchronous neurotransmission release is precisely coupled to action potentials, in contrast, asynchronous release events show only loose temporal coupling to presynaptic activity whereas spontaneous neurotransmission occurs independent of presynaptic activity. The mechanisms that give rise to this diversity in neurotransmitter release modes are poorly understood. Recent studies have described several presynaptic molecular pathways controlling synaptic vesicle pool segregation and recycling, which in turn may dictate distinct modes of neurotransmitter release. In this article, we review this recent work regarding neurotransmitter release modes and their relationship to synaptic vesicle pool dynamics as well as the molecular machinery that establishes synaptic vesicle pool identity.

Introduction: Modes of release and synaptic vesicle pools

The fidelity and plasticity of information flow in neuronal networks relies not only on the speed of neurotransmission but also on its sensitivity to stimuli and changes in neuronal environment. The basic units for neurotransmission are synapses, small intercellular junctions (~1 μm^2) with a narrow intersynaptic space (~15 nm spacing) that allows small neurotransmitters to rapidly diffuse away from the presynaptic terminal and bind to postsynaptic receptors, triggering a response in milliseconds [1,2]. Neurotransmitter release is initiated by action potential (AP) arrival to the axon terminal where it causes a rapid and locally concentrated rise in calcium concentration (called calcium nanodomains [3]) which in turn leads to a simultaneous, time-locked, fusion of synaptic vesicles. This synchronous mode of release constitutes the basis of fast neurotransmission. Synaptic vesicles can also continue to fuse asynchronously for tens to hundreds of milliseconds after the AP, depending on neuron type, developmental stage and prior history of activity [4–8]. The level and duration of asynchronous release is regulated by the extent of calcium entry and the buffering capacity of presynaptic terminals [3], in addition to the intrinsic molecular attributes of the neurotransmitter release machinery (see below). Spontaneous neurotransmitter release, on the other hand, is independent of neuronal activity and exhibits

variable regulation by calcium [9]. These three modes of neurotransmitter release were first discovered and characterized functionally, through electrophysiological approaches, but subsequently it became evident that the molecular properties of synaptic vesicles contribute to this diversity [10].

From a functional perspective, synaptic vesicles can be classified based on their intrinsic propensity to fuse. In this context, the readily releasable pool (RRP) of vesicles are defined as the first line of vesicles that fuse in response to stimulation, i.e. these vesicles have the highest propensity to fuse [11,12]. The RRP is the only pool that has been demonstrated to have a strong function and morphology correlation, [13–16]. In sensory neuron synapses, such as the retinal or auditory ribbon synapses, the number of docked vesicles correlates with the fast, synchronous release triggered by stimulation, while vesicles unrelated to the ribbon tend to fuse spontaneously [13–16]. In small central synapses, including hippocampal synapses, the size of the RRP also corresponds to the number of docked synaptic vesicles at the active zone (AZ) and regulation of AZ size and number of docking and release sites correlates with release probability and synaptic strength [17–19], but also see [20]. The second pool of vesicles that is mobilized and fused in response to APs is the reserve pool, defined as those vesicles that can fuse and take up different probes in response to mild to sustained stimulation, replenishing the RRP and maintaining neurotransmission [21–23]. The sizes of the RRP and the reserve pool are variable among synapses, approximately they occupy ~0.5% and ~10–60% of the total pool, respectively [22,24–26]. The RRP and reserve pool together form the recycling pool of synaptic vesicles. The rest of the synaptic vesicles in the nerve terminal belong to the so called “resting” or “dormant” pool, a group of vesicles highly impervious to activity (they will only fuse after strong high frequency repetitive stimulation, possibly fuse spontaneously or never release at all) [26,27]. The functional role of such a voluminous and largely inactive pool of vesicles has been a subject of speculation for many years. Besides potential contributions to spontaneous release, additional functions may include acting as a reserve for neurotransmitter and synaptic proteins [28,29]. Unlike the RRP, the reserve and resting pools do not show a specific localization within the nerve terminal and vesicles belonging to both pools appear to be intermixed [30], although in some studies actively recycling vesicles have been reported to be distributed closer to the AZ than resting vesicles [20,31]. Moreover, vesicles that are mobilized at different stimulation intensities have been shown to fuse at different sectors of the AZ, where synaptic vesicles that respond to high frequency stimulation fuse at the edges of the AZ [32]. This finding indicates that there are certain spatiotemporal properties that are characteristic of each synaptic vesicle pool.

Molecular basis of release diversity: heterogeneity in synaptic vesicle recycling machinery

Segregation of synaptic vesicle pools appears early in neuronal development, even though the total number of synaptic vesicles is half or less than that of a fully mature synapse. At onset, nascent presynaptic terminals possess an undifferentiated total pool of synaptic vesicles capable of spontaneous recycling as well as evoked Ca^{2+}-dependent release under strong stimulation. Fusion in response to single APs requires the appearance of the RRP

during development, followed by gradual population of the reserve pool [33]. Vesicles that release in a spontaneous or evoked fashion are also separated into different pools during maturation [34]. The main molecular hallmark driving this diversity in release modes of synaptic vesicles is the particular set of fusion machinery proteins present on the vesicle membranes. For instance, the distribution of SNAREs (acronym of Soluble NSF Attachment protein Receptors) and other associated proteins composing the fusion complex among vesicles. Classical SNARE driven fusion of a synaptic vesicle involves docking and priming mediated by the vesicle SNARE synatobrevin 2 (also called vesicle-associated membrane protein 2 or VAMP2) and the plasma membrane SNAREs syntaxin 1 and synaptosomal-associated protein 25 (SNAP-25), which along with the help of the active zone matrix proteins Munc18 and Munc13 assemble and generate a fusion-ready intermediary complex, highly stable and resistant to proteases [35]. Upon AP arrival and rapid Ca^{2+} concentration rise in the terminal, full zippering of the SNARE complex is triggered and membranes are pulled together, catalyzing the fusion and the opening and expansion of the pore. The calcium sensor responsible for fast and cooperative Ca^{2+} sensing and coupling to the fusion process, thus triggering fast, reliable synchronous release, is synaptotagmin 1 (syt1) [36]. These canonical SNAREs are involved in most of evoked synchronous neurotransmission throughout the central nervous system, since deletion of any one of them leads to a drastic decrease in neurotransmission [37–39]. However, a small fraction of synaptic vesicles are still able to fuse, especially those that recycle spontaneously or asynchronously [37,39] indicating that other, non-canonical SNAREs, are involved in these different modes of release (also see [40]). Nevertheless, not all synaptic proteins show specific distribution to a particular pool. Complexins, soluble proteins that selectively bind to SNARE complexes, can differentially regulate synchronous and asynchronous release in various neuronal types, and they also clamp and regulate spontaneous release. Thus, while complexins seem to be broad molecular regulators of all modes of fusion, they can also act in a specific manner for a particular mode of release [41–44], possibly through the interaction with distinct calcium sensors.

Synaptic vesicles that fuse synchronously or asynchronously belong to the same recycling pool [45], but may have different relative content of various SNAREs and Ca^{2+} sensors as result of sustained activity and recycling, generating preferential release modes for each vesicle [46]. The non-canonical vesicular SNARE VAMP4 has been demonstrated to preferentially drive asynchronous fusion and recycling, independently of VAMP2 or complexin and syt1 [46]. Instead of syt1, the calcium sensor for asynchronous release has been shown to be synaptotagmin 7 (syt7) [4,47,48]. Although a molecular interaction between VAMP4 and syt7 has not been experimentally demonstrated, they both recycle with similar kinetics after strong stimulation and seem to partially reside in a plasma membrane pool [46,49–51]. SNAP-25/syt1 complex and SNAP-23/syt7 have been associated with propensity to fuse synchronously and asynchronously, respectively [49], although SNAP-25 and 23 seem to be partially interchangeable. Trafficking differences between syt1 and syt7 containing vesicles may arise from the differences in Ca^{2+} binding properties between the two proteins (affinity, time courses of response) [47], agreeing with the observed differential dependence of synchronous and asynchronous release on Ca^{2+} concentration, buffering speed and source [52–54]. Recently, it was also demonstrated that syt1 and syt7 also couple

release to kinetically distinct routes of endocytosis [51], pointing out that different pools might have independent cycling pathways [55]. Nevertheless, syt1 and syt7 also have partially overlapping, redundant activity [56], supporting imaging and electrophysiological evidence that these two modes of release belong to the same, activity-dependent recycling pool [45,54].

Spontaneously recycling synaptic vesicles are also morphologically indistinguishable from vesicles fusing in response to APs, and are spatially intermixed within the presynaptic terminals [57]. Despite their similar appearance, studies employing functional loading of distinct probes support the notion that spontaneous and evoked vesicle pool identity is conserved after fusion and retrieval, and these pools do not functionally intermix [58–61]. This premise is also supported by the observation that the retrieval kinetics of individual vesicles significantly diverge depending on whether they have initially fused spontaneously or in response to APs [62]. Moreover, the probabilities of release for evoked and spontaneous neurotransmission are not correlated within single presynaptic terminals [61,63]. The spontaneous pool of vesicles recycles at rest and generally do not respond to AP stimulation, although a small proportion can traffic and release upon very intense stimulation, like an elevated (90 mM) K^+ pulse. For that reason, the spontaneously recycling pool has been proposed to overlap with the resting pool of synaptic vesicles [27]. In line with this hypothesis, the non-canonical vesicular SNARE VAMP7 has been shown to be enriched in vesicles belonging to the resting pool [64] and can selectively regulate spontaneous release of neurotransmitters [65]. Additionally, a proportion of the resting synaptic vesicles also carry Vti1a, another non-canonical SNARE that directs them to a more robust spontaneous recycling [66]. Vti1a does not bind to the canonical SNARE complex proteins syntaxin 1 and SNAP-25 [67] and traffics independently of VAMP2 [66]. Instead, it was shown to interact with VAMP4, syntaxin 6 and syntaxin 16 in synaptic vesicles [68] possibly assembling a specific SNARE complex for spontaneous release. In agreement with this premise, besides its apparent role in asynchronous release, VAMP4 can also partially rescue spontaneous neurotransmission in VAMP2 deficient hippocampal neurons [46] suggesting that the division between the different pools is dynamic and depends not on one protein but on a combination of various molecular markers. The mechanisms underlying Ca^{2+} dependence of spontaneous release is intensely debated, but evidence from several laboratories points to a fraction of the spontaneous release being Ca^{2+}-independent and a portion of this pool fusing through a Ca^{2+}-dependent pathway (for detailed review on this topic see [9,69,70]). The Ca^{2+} source for spontaneous neurotransmission may differ compared to evoked release, as it may require release from internal stores [71]. Moreover, about half of all spontaneous fusion events have been shown to be triggered by local Ca^{2+} microdomains generated by stochastic openings of voltage gated Ca^{2+} channels [72], although this regulation seems to be present only in inhibitory synapses [69]. Additionally, extracellular Ca^{2+} concentration has been implicated as a trigger of spontaneous exocytosis through the activation of the Ca^{2+}-sensing G protein–coupled receptor [73], indicating that not one but a number of Ca^{2+}-dependent pathways regulate spontaneous neurotransmission and they might differ among different types of synapses. Potential Ca^{2+} sensors for the coupling of Ca^{2+} to spontaneous release are Doc2 and Doc2 related family of proteins, since their manipulation specifically alters spontaneous

neurotransmission without affecting evoked release [74,75] Doc2 proteins possess C2 domains, similar to synaptotagmins, but they differ in Ca^{2+} affinity and subcellular distribution. In addition, Doc2 proteins are cytoplasmic and associate with membranes in response to Ca^{2+} binding [74] constituting possible switches in Ca^{2+} sensitivity and mobilization of the spontaneous pool of synaptic vesicles. However, it is important to note that some studies proposed that Doc2-mediated spontaneous release is Ca^{2+}-independent [76] thus leaving the question of actual Ca^{2+} sensors for spontaneous neurotransmission still open.

To maintain vesicle pool identities each synaptic vesicle pool needs to be segregated during recycling likely via specific endocytic routes. In this context, the canonical SNARE VAMP2 has been shown to participate in synaptic vesicle reformation (either from the presynaptic plasma membrane or synaptic endosomes) through a clathrin and AP-2 dependent pathway mediated by its interaction with the adaptor proteins CALM and AP-180 [77,78]. Proper sorting of the synaptic vesicle proteins synaptophysin-1 and SV2 is linked to VAMP2 [79] and fast exocytosis-endocytosis coupling is dependent on VAMP2 [80], indicating that trafficking properties of the recycling pool are determined by this canonical vesicular SNARE to a large extent. Interestingly, the Ca^{2+} sensor for synchronous release, syt1 shows a completely independent endocytic pathway [81] facilitated by another clathrin adaptor, stonin 2 [82]. The coexistence of these two parallel pathways might lead to uneven accumulation of different synaptic proteins in synaptic vesicles, generating vesicles with different copy number of syt1 for example, and thus creating vesicles with different synchronicity properties and release probabilities. Endocytic partners for the asynchronous Ca^{2+} sensor syt7 are still unknown, but the non-canonical SNARE for asynchronous neurotransmission, VAMP4 is retrieved to synaptic vesicles through the interaction between its di-leucine motif and the clathrin adaptor AP-1 [83] which seems to be specifically coupled to a bulk endocytosis pathway [84]. Mutations on the di-leucine motif abolish the specificity of VAMP4 dependent pathway recycling and make VAMP4 to traffic similarly to VAMP2 [46]. Remarkably, elimination of stonin 2 (syt1 endocytic partner) from synapses leads to increased bulk endocytosis [82] supporting the notion that synchronous and asynchronous release are coupled to separate endocytic routes [51]. More detailed molecular studies about exo-endocytic complexes is needed to strengthen this hypothesis. VAMP7, which labels largely the resting pool of synaptic vesicles, belongs to the longin family of SNARES (along with VAMP4 but not VAMP2), due to its longer N-terminal region [85]. This region constitutes an auto-inhibitory domain that negatively regulates SNARE complex formation and fusion [86,87] and could explain why VAMP7 containing vesicles are highly resilient to fuse. Moreover, the longing domain of VAMP7 is also necessary to its proper localization through interaction with another clathrin adaptor, AP-3 [88]. Remarkably, the vesicular glutamate transporter (VGluT) possesses numerous endocytic motifs, including several di-leucine motifs, and can interact with AP-1, AP-2 and AP-3, as well as endophilin. Each site seems to be independent and to regulate different VGluT retrieval through separate endocytic pathways [89,90] suggesting that it was evolutionarily selected to be able to traffic to all classes of synaptic vesicles and allow neurotransmitter refilling for all pools. A schematic representation of intermixing of synaptic vesicles with different molecular

identities and the putative SNARE complexes mediating different modes of release is presented in Figure 1.

Besides differential SNARE distribution, other molecular properties of synaptic vesicles have been proposed to be involved in their segregation into distinct pools. Actin interaction seems to be crucial for retrieval and reuse of vesicles belonging to the recycling pool [20,91,92] and association with synapsin was proposed to form a synaptic vesicle matrix that maintains the resting pool and regulates its mobility [93,94]. The active zone cytomatrix scaffold protein RIM has been shown to regulate coupling of synaptic vesicles to voltage gated Ca^{2+} channels and to regulate the size of the RRP via docking and also refilling steps [95,96]. Moreover, RIM and its binding proteins control fast synchronous release [97]. Evoked (synchronous as well asynchronous) release is also dynamin dependent, while fast, single vesicle, spontaneous recycling may not require dynamin [98], leaving an open question about how vesicle membranes are excised after spontaneous fusion. The evoked and spontaneous pools of synaptic vesicles have also differential, opposite membrane cholesterol requirements. Cholesterol appears to facilitate evoked fusion and retrieval of synaptic vesicles while it suppresses spontaneous synaptic vesicle recycling [99,100]. In conclusion, although several molecular markers have been implicated in determining synaptic vesicle pool segregation and maintenance (like the content of particular vesicular SNAREs), the fine tuning and richness of pool diversity seems to involve the concomitant activity of several proteins and lipids, many of them with identities and trafficking properties that remain under investigation.

Closing remarks: impact of neurotransmitter release modes on the physiology and plasticity of neuronal networks

Increasing evidence suggests that synaptic vesicle pool segregation in central synapses is dictated by mechanisms that confer specific trafficking and fusion properties to synaptic vesicles. This vesicle diversity, in turn, is critical for the maintenance of distinct forms of neurotransmitter release. Among the three forms of neurotransmission, synchronous release preserves the precise timing of presynaptic activity given its fidelity to specific activity patterns and precise timing with respect to the arrival of APs. Asynchronous release, on the other hand, complements synchronous release during repetitive activity and maintains neurotransmission strength [45]. In addition, asynchronous release is a crucial regulator of neurotransmission by influencing multiple dynamic parameters including synaptic network recovery (refractory period durations) and synchronicity, reliability and duration of the postsynaptic response, partly through its impact on inhibitory inputs [101–104]. Finally, spontaneous neurotransmitter release regulates synaptic properties including strength and plasticity independent of activity. Spontaneous events also influence dendritic arbor complexity and synapse formation during development [105,106]. Moreover, regulation of synaptic strength by spontaneous events has been implicated in the fast antidepressant effect of ketamine [107–109]. Taken together, different modes of release confer neurons a diversity of plastic responses that can be modulated and combined to transfer and integrate a wide variety of information. Understanding the molecular mechanisms underlying synaptic vesicle pool segregation will not only expand our knowledge of how complex neuronal

networks function, but also generate tools to develop more specific therapeutics for neurological and neuropsychiatric disorders, including depression.

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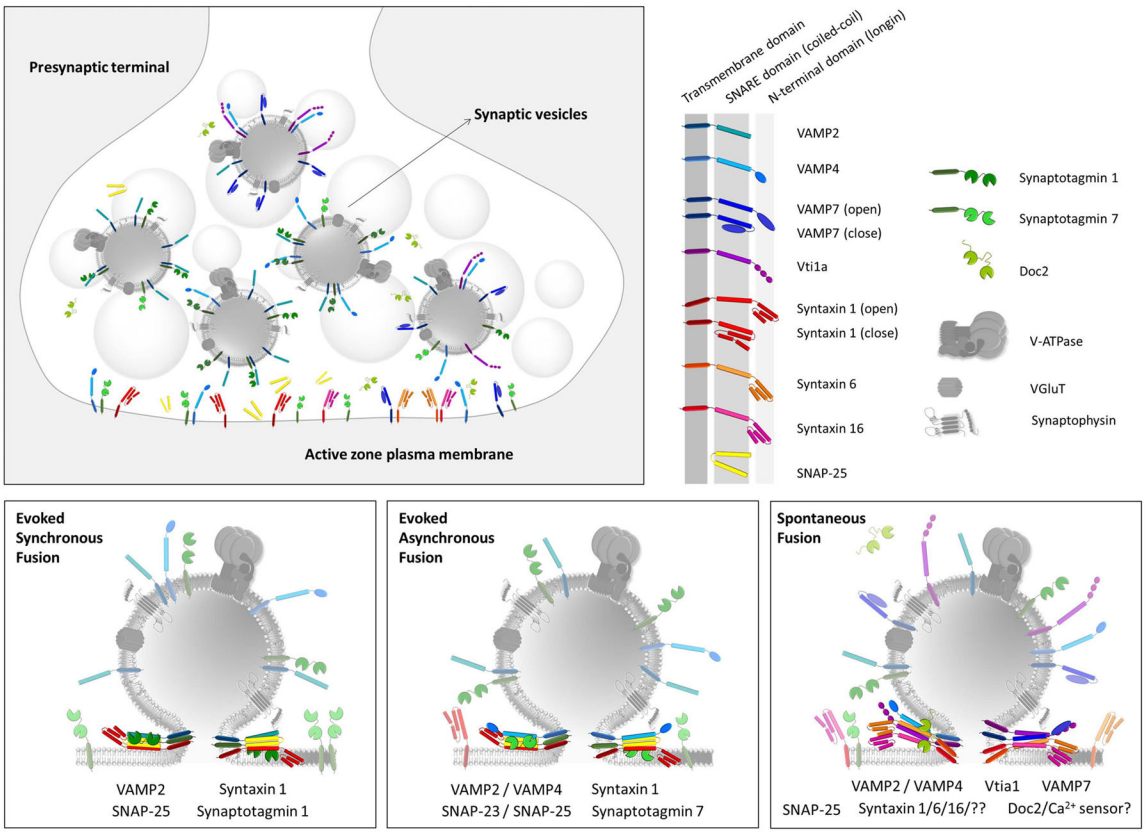

Figure 1.

Top left: Schematic representation of a presynaptic terminal filled with intermixed synaptic vesicles. Recent studies suggest that synaptic vesicles may contain variable combinations of SNARE proteins, as such a vesicle's molecular composition rather than location determines its recycling trajectory.

Top right: Representation of the proteins depicted in the figure. The transmembrane, SNARE and N-terminal (longin) domains of several SNARE proteins are specified.

Bottom: Putative composition of synaptic vesicles and their interaction partners giving rise to the evoked synchronous, asynchronous and spontaneous modes of neurotransmitter release. Although, synchronous fusion requires the canonical SNARE complex composed of VAMP2, SNAP-25 and syntaxin1, vesicles giving rise to asynchronous and spontaneous modes of neurotransmitter release may use a diverse set of SNAREs.