Title:

Synergistic control of neurotransmitter release by different members of the synaptotagmin family.

Short title

Synaptotagmins and vesicular release

Authors

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Abstract

Quantal neurotransmitter release at nerve terminals is tightly regulated by the presynaptic Ca^{2+} concentration. Here, we summarise current advances in understanding how the interplay between presynaptic Ca^{2+} dynamics and different Ca^{2+} release sensors shapes action potential-evoked release on a timescale from hundreds of microseconds to hundreds of milliseconds. In particular, we review recent studies that reveal the synergistic roles of the low Ca^{2+} affinity/fast release sensors synaptotagmins 1, 2 and 9 and the high affinity/slow release sensor synaptotagmin 7 in the regulation of synchronous and asynchronous release and of short-term synaptic plasticity. We also examine new biochemical and structural data and outline a working model that could potentially explain the cooperative roles of different synaptotagmins in molecular terms.

Introduction

Controlled release of neurotransmitters stored in synaptic vesicles (SVs) is central to all information processing in the brain. This process relies on efficient coupling of SV fusion to the triggering signal – action potential (AP)-evoked presynaptic Ca^{2+} influx. SV fusion is catalysed by the synaptic SNARE proteins: VAMP2 (also known as synaptobrevin) on the vesicle membrane (v-SNAREs) and syntaxin and SNAP25 on the pre-synaptic membrane (t-SNAREs)[1-3]. The molecular mechanisms that allow synchronisation of SV fusion to APs remain the subject of debate. AP-evoked release occurs from the readily releasable pool (RRP) of vesicles docked at the presynaptic active zone. A current prevalent view is that on an RRP vesicle the SNARE assembly process is arrested in a 'half-zippered' state, which prevents spontaneous vesicular release but allows for rapid (on a sub-millisecond timescale) SV fusion following AP-evoked Ca^{2+} influx. The key players that 'clamp' SV release and synchronise the late SV fusion steps are complexin (Cpx) and multiple isoforms of the presynaptic Ca^{2+} release sensor synaptotagmin (for recent reviews see [3-7]).

In this review, we focus on the emerging concept that different modes of AP-evoked release are synergistically regulated by several synaptotagmin isoforms with distinct molecular properties. Spontaneous release, as well as the roles of the other parts of SV

fusion machinery and of the vesicular pool dynamics are discussed in several recent reviews [4;6-8], including the review by Chanaday and Kavalali [9] in this issue.

Synaptic functions of the low affinity Ca^{2+} sensors Syt1, Syt2 and Syt9

It is well established that the fast synchronous component of SV release is triggered by Ca^{2+} binding to the low affinity sensors Syt1, Syt2 or Syt9 [10-13]. Synchronous release occurs on a millisecond timescale in response to AP-evoked activation of presynaptic voltage-gated Ca^{2+} channels (VGCCs) that are closely coupled (<100 nm) to release-ready SVs docked at the AZ [14;15]. Opening of VGCCs leads to the formation of local transient (~ 1 – 2 ms) Ca^{2+} nano/microdomains ($[Ca^{2+}]_{local}$) reaching ~ 10 – 100 µM, followed by activation of the low affinity synaptotagmins. The Syt1, Syt2 and Syt9 isoforms are SV-associated proteins, with a large cytoplasmic part, containing tandem C2A and C2B Ca^{2+}-binding domains [6;12;16;17]. Upon Ca^{2+} binding, the adjacent surface loops on each of the C2 domains partially insert into the membrane [18-20] and this membrane insertion is critical for the triggering of SV fusion [21-23]. Whilst Ca^{2+} binding to the C2A domain has been shown to assist in Ca^{2+}-triggering of exocytosis, the activation of the C2B domain is absolutely essential. Indeed, disrupting Ca^{2+} binding or membrane loop insertion of this domain completely abolishes synchronous release [24-28]. Syt1 binds the SNARE proteins via its C2B domain and these interactions are also critical for triggering synchronous release [29-31]. The precise sequence of molecular events that leads to fast SV fusion after Ca^{2+} binding to Syt1 however remains enigmatic.
In addition to the ablation of synchronous release, genetic elimination of Syt1 or Syt2 leads to a several-fold increase in the rate of spontaneous release (detected postsynaptically as "miniature" quantal events) [10;13;25;32]. This implies that synaptotagmins normally clamp SV exocytosis in the absence of APs. Mutational analysis indicates that this clamping function is molecularly distinct from the Ca^{2+} activation of fast SV exocytosis [6;26;32]. Syt1, Syt2 and Syt9 can all activate the fast synchronous release component (with different rates accounting for specific kinetics of synchronous release in different synapses *e.g.* refs. [11;33]), but their clamping abilities may vary [34]. Interaction of Syt1 with the presynaptic membrane under basal conditions via its C2B domain have also been implicated in SV docking at the active zone [31;35].

Role of Syt7 in mediating asynchronous release

In addition to fast synchronous release, which keeps pace with APs, many synapses also exhibit a slower delayed component of AP-evoked release. This asynchronous release is triggered by a persistent elevation in free Ca^{2+} in the presynaptic terminals ($[Ca^{2+}]_{residual}$ ~ 1 – 5 µM) that decays slowly over tens or even hundreds of milliseconds after an AP (for recent review see [7]). The high affinity Ca^{2+} sensors responsible for the asynchronous release component are however much less characterised. Recent studies point to a major role for Syt7, which binds Ca^{2+} in the low micromolar range [36-41] (**Table 1**). Syt7 is abundantly expressed in the brain and is evolutionarily conserved. In neurons Syt7 is not found in SVs and is primarily located to the plasma membrane [42;43]. Initial studies using Syt7 mouse knockout (KO) models failed to reveal a neuronal phenotype [44] or a detectable effect of Syt7 elimination on transmitter release in cultured GABAergic synapses [45]. It was however later demonstrated that Syt7 does mediate the asynchronous release component in Syt1 deficient neurons [37]. In that study, the authors showed that Syt7 knockdown (KD) selectively suppresses asynchronous release in response to single APs or high frequency AP bursts in both

glutamatergic and GABAergic synapses in primary neuronal cultures that lack Syt1. Further rescue experiments demonstrated the critical importance of Ca^{2+} binding by the Syt7 C2A domain in mediating asynchronous release, in contrast to the dominant role of the Syt1 or Syt2 C2B domain in triggering synchronous release. Taken together these findings suggest that multiple synaptotagmin isoforms cooperate at a given synapse in triggering different modes of Ca^{2+}-dependent transmitter release. Because asynchronous release is potentiated in the absence of Syt1 [26;32;46;47], it is likely that under physiological conditions Syt1 acts as a clamp that gates Syt7-mediated asynchronous release. The rate of asynchronous release dramatically increases during high frequency bursts of APs due to an increase in residual presynaptic Ca^{2+} [7;38;40;48] and possibly due to progressive release of the Syt1 (or Syt2) clamp. Such asynchronous release in the intervals between APs during high frequency stimulation is markedly reduced in Syt7 deficient synapses even in the presence of Syt1 or Syt2, further confirming the role of Syt7 as the major Ca^{2+} sensor for asynchronous release [37-40].
The delayed release that occurs at many synapses after AP bursts, lasting several hundred milliseconds, is implicated in prolonged synaptic computations [7;48;49]. Notably, the Syt7 KO mouse model revealed an unexpected physiological role for asynchronous release at synapses that lack such a delayed release phase. In a recent study, Lou and Sudhof showed that in the calyx of Held synapse (which is specialised for fast information transfer) asynchronous Syt7-mediated release contributes a steady-state postsynaptic current component during high frequency stimulation. This facilitates reliable generation and temporal precision of postsynaptic APs that are time-locked to presynaptic spikes [38]. A similar contribution of asynchronous release to the precision of postsynaptic AP spiking was also reported at the hippocampal mossy fibre–CA3 pyramidal cell synapse[50].
It is important to note that genetic deletion of Syt7 does not completely abolish the asynchronous release, pointing to a contribution from other Ca^{2+} release sensors. A cytosolic C2AB domain-containing protein Doc2A has been implicated [51], but several other studies have argued against a role for Doc2 proteins in asynchronous release [37;52;53]. It also remains to be determined to what extent the low affinity Ca^{2+} sensors Syt1, 2 and 9 contribute to asynchronous release in physiological conditions.

Syt7 as a Ca^{2+} sensor for short-term synaptic facilitation.

As discussed above, low affinity Ca^{2+} sensors Syt1 and Syt2 can gate the Syt7-mediated asynchronous release component. Conversely, it is emerging that the high affinity sensor Syt7 can modulate synchronous release mediated by Syt1 or Syt2. Indeed, several recent studies reported a major role of Syt7 in the regulation of short-term synaptic plasticity of fast synchronous release. It has been demonstrated that Syt7 KO results in a profound reduction in short-term facilitation and/or an increase in short-term depression at different types of glutamatergic and GABAergic synapses (**Table 1**) [40;41;54;55].
Short-term facilitation at Syt7 KO synapses could be rescued by overexpression of wild type Syt7 but not by Syt7 with C2A Ca^{2+}-binding deficient mutations [40;54;55]. This further demonstrates a key functional role of Syt7 C2A domain. Importantly, the effect of Syt7 deletion on short-term plasticity was not due to changes in initial release probability or presynaptic Ca^{2+} dynamics [41;54]. It has been proposed that Ca^{2+}-triggered membrane insertion of the Syt7 C2A domain during repetitive stimulation is likely to reduce the overall energy required for fusion and in this way facilitate Syt1- (or Syt2-) mediated synchronous release [56]. This further argues that the two types of

release sensors cooperate in triggering AP-evoked exocytosis of the same SVs. At some synapses Syt7 also contributes to shaping synaptic transmitter release during prolonged stimulation via Ca^{2+}-dependent synaptic vesicle replenishment and by maintaining the RRP size [40;57].
In contrast to the effects of deleting Syt7 described above, short-term synaptic plasticity was not altered at GABAergic synapses in Syt7 KO cultures [45] or at young calyx of Held synapses [38]. It would be interesting to establish to what extent the synapse specific effects of Syt7 KO on short-term facilitation and on asynchronous release are determined by the relative expression levels of multiple synaptotagmin isoforms and their splice variants in different neuronal types.

Ca^{2+}-dependent membrane interaction properties of Syt1 and Syt7 C2 domains

Can the specific roles of Syt1 (Syt2 and Syt9) and Syt7 in the regulation of AP-evoked release be explained by differences in Ca^{2+} binding and membrane interaction of their C2 domains? The biochemical and structural properties of the C2A and C2B domains of Syt1 and Syt7 have been extensively characterised. In solution, the C2A and B domains of both Syt1 and Syt7 show similar intrinsic Ca^{2+}-affinities (≥ 100 µM) as determined using isothermal titration calorimetry (ITC) [36;58] (**Figure 1A**). In the presence of liposomes that contain acidic lipids (25% phosphatidylserine, PS) synaptotagmin molecules can interact with membranes both independently of Ca^{2+} (via the polybasic motif of the C2B domain [19;59-61] and via Ca^{2+}-dependent insertion of aliphatic loops [18-20]. Under these conditions both C2A and B domains of Syt1 and Syt7 exhibit higher apparent Ca^{2+}-affinity, but Syt7 C2domains have ~10 fold higher affinity than Syt1 C2 domains [62], demonstrating stronger Ca^{2+}-dependent membrane association of Syt7. Correspondingly, under saturating [Ca^{2+}] conditions Syt7 exhibits a higher apparent affinity for PS-containing membranes as compared to Syt1 [36]. Inclusion of Phosphatidylinositol 4,5-bisphosphate (PIP2), which strongly enhances Ca^{2+}-independent membrane binding of C2B domains [19;60], also increases the overall membrane affinity of both Syt1 and Syt7 (**Figure 1B**). In all cases Syt7 exhibits much stronger Ca^{2+}-dependent membrane binding than Syt1. Thus, the overall Ca^{2+}-sensing properties of Syt1 and Syt7 correlate with their functions, *i.e.* Syt1 is activated only by high $[Ca^{2+}]_{local}$ (~ 10 – 100 µM) during AP-evoked VGCC opening, whilst Syt7 can also operate during longer lasting increase in residual Ca^{2+} in the low micromolar range.
Additionally, the relative Ca^{2+}-dependent membrane binding properties of the Syt1 and Syt7 C2 domains correlate with the dominant roles of the Syt1 C2B domain in triggering synchronous release and of the Syt7 C2A domain in mediating asynchronous release and short-term plasticity. In the presence of PIP2 the C2B domain of Syt1 shows stronger membrane association in comparison to the C2A domain. In contrast, the C2A domain of Syt7 has tighter membrane binding than its C2B domain irrespective of membrane composition (**Figure 1B**).
The tighter Ca^{2+}-dependent membrane binding of Syt7 C2A domain has been attributed to the stabilisation of its membrane-inserted aliphatic loops [36]. In line with this, the Syt7 C2A domain exhibits significantly slower membrane dissociation kinetics compared to Syt1 (~20-60 fold) in rapid-mixing stopped-flow experiments [63;64]. In fact, the Syt7 dissociation rate constant which is in the order of hundreds milliseconds, agrees well with the time course of Syt7-dependent short-term facilitation [56]. On the other hand, the Ca^{2+}-dependent membrane association rates of the Syt7 C2A domain and of both Syt1 C2 domains are of a similar order (160 s^{-1} and ~ 250 – 350 s^{-1} respectively) [64-66] (**Figure 1C**). Considering that the Ca^{2+}-dependent membrane insertion of C2

domains acts as the molecular trigger for activation of SV fusion, this ~ 2-fold difference in the membrane association kinetics of Syt1 and Syt7 cannot on its own explain the observed 10 –100 fold differences in the rates of synchronous and asynchronous release [48]. Therefore, other factors, including differential interactions with the rest of the synaptic fusion machinery and the spatial organisation at the release sites, must be a key contributing factor.

Possible structural basis for synergistic action of Syt1 and Syt7

In recent years, considerable progress has been made in understanding the structural organisation of synaptic release sites [29;30;67-72]. Of particular interest is the recent high-resolution crystal structure of the primed Syt1-Cpx-SNARE complex from the Brunger and Sudhof laboratories [30]. This structure reveals that there are two Syt1 binding sites on each primed SNARE complex – the Cpx independent 'primary' site which is uniquely found in synaptotagmin isoforms that mediate fast synchronous release (Syt1, Syt2 and Syt9) and the Cpx-dependent 'tripartite' site which is conserved in all synaptotagmin isoforms, including Syt7 (**Figure 2**). Syt1 mutations that disrupt either 'primary' or 'tripartite' site interactions *in vitro* impair the ability of these mutants to rescue synchronous transmitter release and to maintain the RRP of vesicles in cultured neurons lacking endogenous Syt1 and Syt7 [30]. This indicates the importance of both sites in mediating SV release.
Notably, Syt7 contains only the 'tripartite' binding motif whilst Syt1 can bind SNAREpins at both the 'primary' and the 'tripartite' sites. This suggests a parsimonious model for how Syt1 and Syt7 can synergistically regulate SNARE-dependent SV fusion, with Syt1 C2B domains occupying the 'primary' site and with Syt1 and Syt7 C2B domains competing for the 'tripartite 'site (**Figure 3**). According to this model, there are two populations of clamped SNAREpins on a release-ready vesicle: one with Syt1 on both binding sites and the other with Syt1 and Syt7. The model further assumes that to release a SNAREpin from this basal 'clamped' state synaptotagmins at both 'primary' and 'tripartite' sites need to be activated by Ca^{2+}. A given RRP vesicle contains multiple half-zippered SNAREpins and it has been reported that simultaneous activation of several SNAREs is required for vesicle fusion (in the range of two to eight) [73-75]. Furthermore experiments with constitutively open mutation of the t-SNARE syntaxin showed that SV fusion rate increases with the number of activated SNAREpins [76]. Thus, time-dependent activation of multiple SNAREpins of different types is likely to determine the temporal profile of vesicular release probability during AP-evoked presynaptic Ca^{2+} transient. During formation of fast AP-evoked high nano/microdomain $[Ca^{2+}]_{local}$ only the SNAREpins primed with two Syt1 molecules will be activated by Ca^{2+} and contribute to synchronous release. The slower Ca^{2+} binding by Syt7 will delay the removal of the clamp from mixed Syt1/Syt7 SNAREpins thus supporting asynchronous release. Relatively fast dissociation of Ca^{2+} from Syt1 C2B domains will restore the clamp on the 'primary' site and in this way will gate Syt7-mediated asynchronous release. Finally, persistence of the Ca^{2+}-activated state of the Syt7 C2A domain will allow facilitation of synchronous release during repetitive stimulation. The proportions of Syt1/Syt1- and Syt1/Syt7-SNAREpins on RRP SVs are likely to be determined by the expression levels of Syt1 (Syt2, Syt9) and Syt7. This can potentially explain the observed differences in the synchronous to asynchronous release ratio among different synapse types. Furthermore, the relative abundance of Syt1/Syt1- and Syt1/Syt7-SNAREpins possibly varies even among vesicles in the same presynaptic bouton [9;77]. Notably, initial probability of synchronous release is not changed in Syt7 KO [54], indicating that the number of

Syt1/Syt1-SNAREpins is not affected by the elimination of Syt7. This implies that formation of Syt1/Syt1-SNAREpins could be independent of Syt7. On the other hand it is entirely possible that Syt7 could be replaced by the other presynaptic C2AB domain-containing proteins (e.g. Doc2) that may also contribute to the dynamic regulation of vesicular SNAREpins via the 'tripartite' binding side [30]. Although highly speculative, the above model provides a plausible framework to describe the cooperative action of different synaptotagmins in AP-evoked release in molecular terms.

SV release exhibits a steep power dependency on [Ca^{2+}]. This implies that several Ca^{2+} ions are required to bind to one or more synaptotagmin molecules to trigger SV fusion [78;79]. Furthermore, energetic considerations argue that synchronous activation of several SNARE complexes on the same vesicle is required to sustain the rate of fast synchronous SV fusion [76;80]. How can the action of several synaptotagmins and SNARE complexes be coordinated? One way to achieve this is via a high-order structural organisation that could link multiple synaptotagmin-SNARE complexes together. A possible solution comes from a recently discovered conserved structural feature of C2 domain-containing proteins (including Syt1, Syt1 and Syt7) whereby they form Ca^{2+}-sensitive oligomeric structures *in vitro* [70;81;82]. The oligomerisation of Syt1 is mediated by its C2B domain and is triggered by the binding of polyvalent anions (*e.g.* ATP in solution or PS/PIP2 on membranes) to its conserved poly-lysine motif [70;81;82]. Formation of Syt1 oligomers is compatible with SNARE binding via the primary site [82]. It has been proposed that if the Syt1 oligomeric structure were to form at the site of SV docking, it could then act as a template for multiple SNAREpin formation and in this way synchronize the force of many SNAREs co-operatively to release explosively [67;70]. At the same time, the Syt1 oligomers bound to the 'primary' site would physically block the complete assembly of the SNARE complexes and spontaneous SV fusion under basal conditions. Although at present there is no evidence for such oligomeric Syt1 structures in synapses, synaptotagmin oligomerisation represents an attractive model to explain the kinetics of Ca^{2+}-triggered SV fusion.

Concluding remarks

Taken together the recent functional and structural data discussed here argue that different modes of synaptic neurotransmitter release are synergistically regulated by multiple synaptotagmin isoforms. The differential expression and targeting of synaptotagmins with distinct biochemical properties is likely to account for the fine tuning of Ca^{2+}-evoked neurotransmission at different types of synapses.

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Table1

Effects of Syt7 KO and KD on asynchronous release and short-term plasticity in different synapses

Synapse type	**Asynchronous release**	**Short-term facilitation**	**Refs.**
GABAergic and glutamatergic synapses in primary hippocampal cultures	Strongly reduced by Syt7 KO or KD on Syt1 KO background, can be rescued by Syt7 WT but not Syt7 C2A* Ca^{2+}-deficient mutant. In the presence of endogenous Syt1 inhibited by ~50% during high frequency stimulation.	No apparent effect in Syt7 KO or KD.	[37]
Hippocampal Schaffer collateral synapses between hippocampal CA3 and CA1 pyramidal cells	Not determined (ND)	Short-term facilitation is abolished in Syt7 KO, can be rescued by Syt7 WT but not Syt7 C2A* Ca^{2+}-deficient mutant.	[54]
Mossy fibre synapses between hippocampal dentate granule and CA3 cells	ND	Reduced in Syt7 KO	[54]
Thalamocortical synapses between layer VI cortical pyramidal cells and thalamic relay cells	ND	Abolished in Syt7 KO	[54]
Perforant path synapses between layer II and III cells of the entorhinal cortex and dentate granule cells	ND	Abolished in Syt7 KO	[54]
Calyx of Held, P11-P14	Strongly reduced during 100Hz stimulation in Syt7 KO and in Syt2/7 DKO	No detectable effect in Syt7 KO, blocked short-term facilitation in Syt2/7 DKO	[38]
Synapses from Purkinje cells to deep cerebellar nuclei	ND	Syt7 mediates facilitation that is normally occluded by depression, which can be revealed in wild-type mice but is absent in Syt7 KO mice, can by rescued by Syt7 WT	[55]
Vestibular synapses in magnocellular medial vestibular nucleus	ND	Syt7 mediates facilitation that is normally occluded by depression, which can be revealed in wild-type mice but is absent in Syt7 KO mice	[55]
Synapses from basket cells on to Purkinje cells in cerebellum	Reduced during high frequency stimulation in Syt7 KO	Abolished in Syt7 KO	[40]
Synapses from granule cells onto stellate cells and onto Purkinje cells in cerebellum	Reduced in Syt7 KO	Reduced in Syt7 KO	[41]

Figure legends

Figure 1. The Ca^{2+} and membrane binding properties of Syt1 and Syt7. (**A**) In solution, C2AB domains of Syt1 and Syt7 bind 2-3 Ca^{2+} with similar intrinsic affinity, as measured by isothermal titration calorimetry[36;58]. (**B**) The presence of membranes increases the apparent Ca^{2+} affinity of both Syt1 and Syt7 as the C2 domains can interact with acidic lipids (DOPS and PIP2) via polylysine motif (Ca^{2+}-independent binding) and via aliphatic loop insertion (Ca^{2+}-dependent binding). In these conditions Syt7 exhibits a higher apparent Ca^{2+} affinity than Syt1, as estimated by co-sedimentation analysis with

liposomes containing 25% DOPS carried under non-saturating [Ca^{2+}] [62]. Correspondingly, Syt7 also shows a stronger membrane affinity under saturating free [Ca^{2+}] (1 mM) as measured by FRET-based membrane interaction assay with liposomes containing 12% DOPS or 12% DOPS + 1% PIP2 [36]. (**C**) The higher Ca^{2+}/membrane affinity of Syt7 is primarily due to its slower dissociation kinetics from membranes (~20-60 fold difference in k_{off} compared to Syt1), whilst the Ca^{2+}-induced membrane association kinetics (k_{on}) differ less than 2 fold. The membrane association/dissociation rates were determined by FRET-based stopped flow measurements following rapid mixing of Ca^{2+} to Syt1/Syt7 fragments in the presence of DOPS liposomes (association) or EGTA to Ca^{2+}/membrane bound Syt1/Syt7 (dissociation) [63;64].

Figure 2. Two synaptotagmin molecules can bind to a single SNARE complex. Crystal structure of the pre-fusion Syt1-Cpx-SNARE complex showing the presence of two distinct binding sites for Syt1 on the opposite surfaces of a SNAREpin [30]. Both Syt1 interactions are mediated by its C2B domain: (i) Cpx-independent interfacing with the SNAP25 helices (green), termed 'primary' binding site; (ii) Cpx-dependent, involving portions of helices derived from complexin (cyan), syntaxin (red) and VAMP2 (blue), termed the 'tripartite' binding site. The 'primary' interface is exclusively found on the low Ca^{2+} affinity fast release sensors (Syt1/2/9) whilst the 'tripartite' binding site is conserved among all synaptotagmin isoforms and several other C2 domain proteins.

Figure 3. Molecular model of cooperative activation of SNARE-dependent SV fusion by Ca^{2+} binding to Syt1 and Syt7.

The model is based on the recent high-resolution crystal structure of Syt1-Cpx-SNARE complex shown in Figure 2 [30]. It assumes that the 'primary' site on a given SNAREpin can only bind Syt1 C2B domain, whilst the 'tripartite' site can interact either with Syt1 C2B or Syt7 C2B domains. Thus, there are two populations of SNAREpins on a RRP vesicle: Syt1/Syt7- and Syt1/Syt1-bound. The model also assumes that the release of a given SNAREpin from the 'clamped' state only occurs when synaptotagmins on both 'primary' and 'tripartite' are activated by Ca^{2+}. For simplicity, here we only consider critical Ca^{2+} activation of Syt1 C2B and Syt7 C2A domains. Time dependency of [Ca^{2+}] at a typical release site in response to a pair of APs is schematically shown at the top. During fast transient increase of [Ca^{2+}] (~1 ms, ~ 10 – 100 μM) immediately after the first AP Ca^{2+} binding by Syt1 C2B domains predominantly activates Syt1/Syt1-SNAREpins, which allows synchronous release. The slower Ca^{2+} binding by Syt7 delays the removal of the clamp from mixed Syt1/Syt7 SNAREpins leading to asynchronous release (> 10 ms timescale). Ca^{2+} dissociation from Syt1 C2B domains restores the clamp on the 'primary' site and in this way inhibits Syt7-mediated asynchronous release. Finally, the persistence of the Syt7 C2A domain Ca^{2+}-activated state leads to facilitation of synchronous release at the second APs by increasing the number of activated SNAREpins.

Highlighted references

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[30]

Zhou and colleagues provide the first high-resolution structure of the Syt1-complexin primed SNARE complex. They identify two distinct binding sites for Syt1 on the pre-fusion SNARE complex: a complexin-independent 'primary' binding site and complexin-dependent 'tripartite' site. They use targeted mutations in Syt1 and SNAREs to establish the critical importance of both binding sites for AP-evoked neurotransmitter release.

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[36]

In this article, the authors combine X-ray crystal structure analysis, with biophysical and biochemical experiments to demonstrate that the higher apparent Ca^{2+}-affinity of Syt7 is due to the exceptionally tight binding of Syt7 to lipid membrane. They further establish the critical role of the C2A domain in this process and argue that the tighter membrane binding is due to the subtle differences in the membrane-inserted aliphatic residues.

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[37]

In this article, the authors demonstrate that Syt7 plays a major role in mediating asynchronous release in mammalian synapses. They show the critical importance of Ca^{2+} binding by Syt7 C2A domain as well as the role of Syt1 in clamping of Syt7-driven asynchronous release.

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[38]

In this study the authors expand the physiological role for asynchronous release in synapses specialised for fast information transfer, such as the calyx of Held. They demonstrate that Syt7-mediated asynchronous release produces a basal current during spike trains that boosts synchronous transmission to enable high fidelity rapid information transfer.

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[40]

Using knockout model the authors demonstrate that Syt7 has a triple function at cerebellar inhibitory synapses and acts as a Ca2+ sensor for asynchronous release, short-term facilitation and vesicle pool replenishment. The authors further show that Syt7 facilitates the efficacy and frequency independence of inhibitory synaptic transmission at this synapse.

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[54]

Using knockout model the authors demonstrate critical importance of Syt7 in mediating short-term synaptic facilitation in four different types of central synapse. They also show the key role of Syt7 C2A domain using rescue experiments.

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[55]
In this study the authors demonstrate that Syt7 regulates short-term synaptic plasticity in depressing synapses from Purkinje cells to deep cerebellar nuclei and at vestibular synapses in mice. They show that Syt7 supports facilitation that is normally masked by depression at these synapses, which can be revealed in wild-type mice but is absent in Syt7 knockout mice.

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[70]
Using negative stain electron microscopy, Wang and colleagues identify the intrinsic property of Syt1 molecules to oligomerise into Ca^{2+}-sensitive ring-like structures on lipid surfaces. They establish a critical role of the C2B domain in both the oligomer assembly and its Ca^{2+} sensitivity, and propose a novel mechanism by which the Syt1 oligomers could regulate neurotransmitter release.

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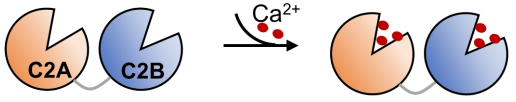
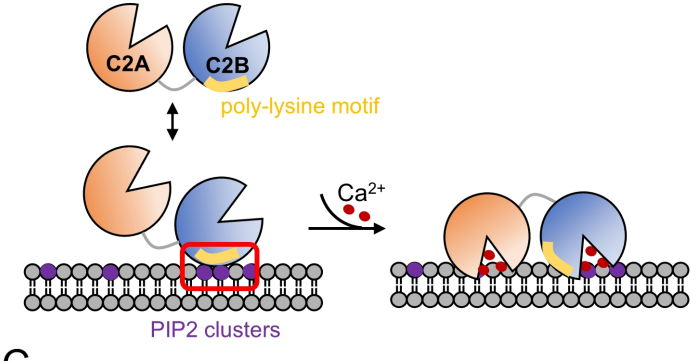
A

In the absence of lipids



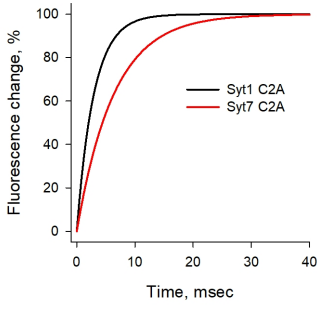
B

In the presence of lipids (DOPS/PIP2)

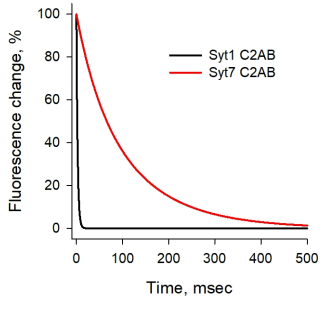


C

Ca2+-triggered membrane association



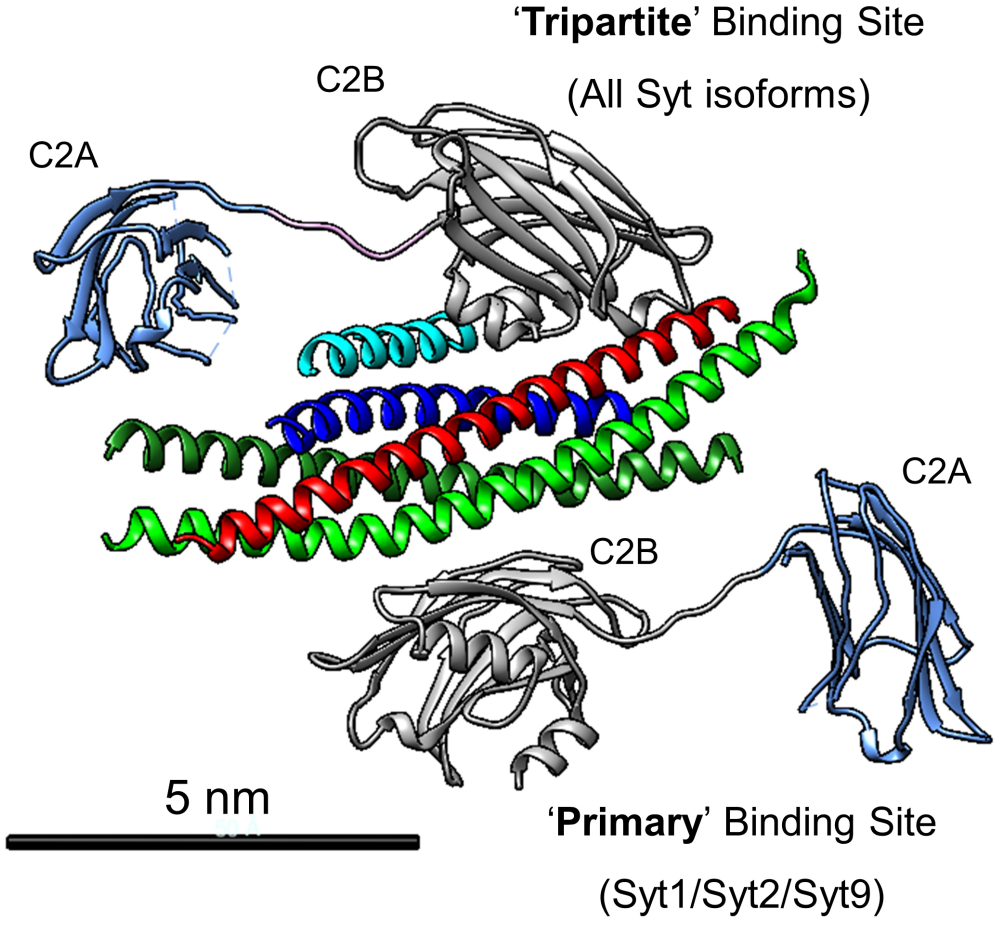
EGTA-triggered membrane dissociation

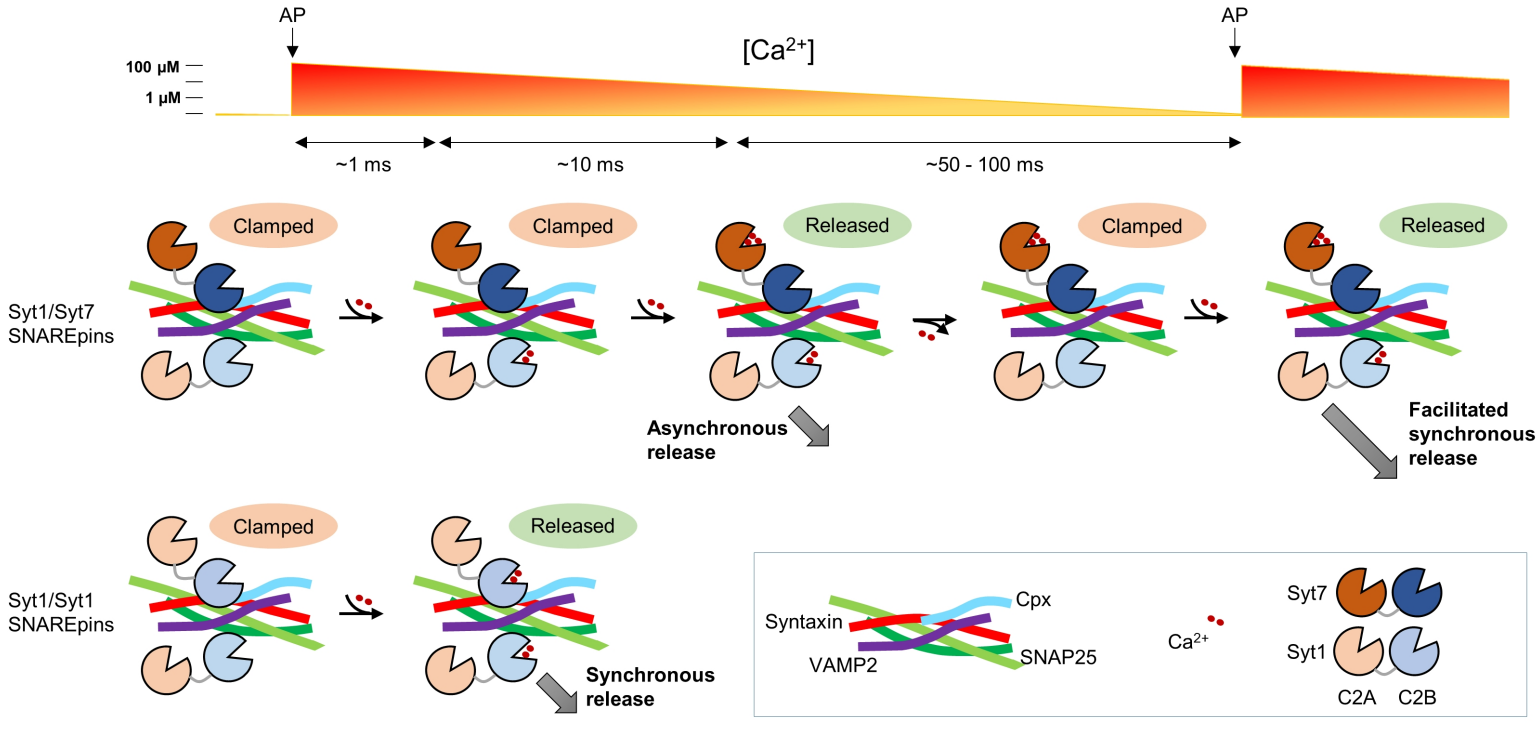


Intrinsic Ca^{2+} Affinity (in absence of lipid membranes)			
C2A domain	Syt1	~120 μM, ~465 μM, ~1.7 mM	Radhakrishnan *et al.* 2009
	Syt7	~120 μM; ~180 μM; ~11 mM	Voletti et al., 2017
C2B domain	Syt1	~200 μM	Radhakrishnan *et al.* 2009
	Syt7	75-900 μM, 0.5-1.2 mM, 3-5 mM	Voletti et al. 2017

		Apparent Calcium Affinity Non-saturating $[Ca^{2+}]$ (Sugita *et al.* 2012)	Apparent Membrane Affinity Saturating $[Ca^{2+}]$ (Voletti *et al.*, 2017)	
		DOPS	*DOPS*	*DOPS/PIP2*
C2A domain	Syt1	~10 μM	~500 μM	~500 μM
	Syt7	~1.5 μM	~2.5 μM	~2.5 μM
C2B domain	Syt1	~20 μM	~635 μM	~10 μM
	Syt7	~2.5 μM	~35 μM	~5 μM

	Association Constant (k_{on}) DOPS membrane	Dissociation Constant (k_{off}) DOPS membrane
Syt1	~220 -340 s^{-1} (Davis *et al.* 1999, Brandt *et al.* 2012, Krishnakumar *et al.* 2013)	~380-670 s^{-1} (Hui *et al.* 2005, Brandt *et al.* 2012)
Syt7	~160 s^{-1} (Brandt *et al.* 2012)	~11-20 s^{-1} (Hui *et al.* 2005, Brandt *et al.* 2012)


‘**Tripartite**’ Binding Site
(All Syt isoforms)
C2B
C2A
C2A
C2B
5 nm
‘**Primary**’ Binding Site
(Syt1/Syt2/Syt9)


AP
AP
[Ca2+]
100 µM
1 µM
~1 ms
~10 ms
~50 - 100 ms
Syt1/Syt7
SNAREpins
Clamped
Clamped
Released
Clamped
Released
Asynchronous
release
Facilitated
synchronous
release
Syt1/Syt1
SNAREpins
Clamped
Released
Synchronous
release
Syntaxin
Cpx
VAMP2
SNAP25
Ca2+
Syt7
Syt1
C2A
C2B