

# Intracellular Ca<sup>2+</sup> release and synaptic plasticity - a tale of many stores

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## Abstract

Ca<sup>2+</sup> is an essential trigger for most forms of synaptic plasticity. Ca<sup>2+</sup> signalling occurs not only by Ca<sup>2+</sup> entry via plasma membrane channels, but also by Ca<sup>2+</sup> signals generated by intracellular organelles. These organelles, by dynamically regulating the spatial and temporal extent of Ca<sup>2+</sup> elevations within neurons, play a pivotal role in determining the downstream consequences of neural signalling on synaptic function. Here, we review the role of three major intracellular stores: the endoplasmic reticulum, mitochondria, and acidic Ca<sup>2+</sup> stores, such as lysosomes, in neuronal Ca<sup>2+</sup> signalling and plasticity. We provide a comprehensive account of how Ca<sup>2+</sup> release from these stores regulates short- and long-term plasticity at the pre- and postsynaptic terminals of central synapses.

## Introduction

Ca<sup>2+</sup> is an essential trigger for many forms of synaptic plasticity. Indeed, it is well understood how Ca<sup>2+</sup> influx from the extracellular environment, specifically via N-methyl D-aspartate receptors (NMDARs) and voltage-gated Ca<sup>2+</sup> channels (VGCCs), shape short- and long-term changes, both pre- and postsynaptically (Blundon & Zakharenko, 2008; Bliss & Collingridge, 2013; Padamsey & Emptage, 2014; Padamsey and others, 2017c). However, in addition to external sources of Ca<sup>2+</sup>, there exist a number of intracellular Ca<sup>2+</sup> stores, which comprise of the endoplasmic reticulum (ER), mitochondria, and acidic Ca<sup>2+</sup> stores, including lysosomes, lysosome-related organelles, and endosomes. These stores play a pivotal role in shaping the spatial temporal dynamics of Ca<sup>2+</sup> signalling. They are capable of initiating rapid and localized Ca<sup>2+</sup> *sparks*, orchestrating cell-wide Ca<sup>2+</sup> *waves*, and regulating tonic cytosolic Ca<sup>2+</sup> levels (Ross, 2012). Such signalling has complex effects on activity-dependent plasticity, and

consequently, investigations of these effects can yield conflicting and varied results. Nevertheless, despite the complexity, a common logic exists in how  $\text{Ca}^{2+}$  stores govern synaptic changes. In this review we focus predominantly on hippocampal CA3-CA1 synapses, where  $\text{Ca}^{2+}$  signalling and plasticity have been extensively studied, and attempt to resolve discrepant findings across the literature when they emerge. Our aim is to provide a comprehensive and unified account of store-dependent  $\text{Ca}^{2+}$  signalling, and how it impacts both pre- and postsynaptic plasticity (summarized in Table 1). Across the literature, we find three general principles: 1)  $\text{Ca}^{2+}$  stores are dynamic and relocate in an activity-dependent manner. 2) Presynaptic stores largely influence presynaptic plasticity, and postsynaptic stores largely influence postsynaptic plasticity. 3) The extent to which a store is involved in regulating synaptic function and plasticity critically depends upon the pattern of neuronal activity.

### **The Endoplasmic Reticulum**

The smooth ER network represents a spatially contiguous organelle that can be found throughout neurons. ER-bound  $\text{Ca}^{2+}$ ATPases (SERCAs) concentrate  $\text{Ca}^{2+}$  within the lumen of the organelle to about 500  $\mu\text{M}$ , approximately three orders of magnitude greater than resting cytosolic  $\text{Ca}^{2+}$  levels (Meldolesi & Pozzan, 1998; Mogami and others, 1998; Shen and others, 2011). ER  $\text{Ca}^{2+}$  release is mediated by two major class of receptors: 1) ryanodine receptors (RyRs), which are activated by cytosolic  $\text{Ca}^{2+}$  in concert with cyclic ADP ribose (cADPR) (Lanner and others, 2010), and 2) the inositol trisphosphate receptors ( $\text{IP}_3$ Rs), which are activated by cytosolic  $\text{Ca}^{2+}$  in the presence of  $\text{IP}_3$  (Taylor & Tovey, 2010).

#### *Postsynaptic RyRs mediate $\text{Ca}^{2+}$ release in dendrites and spines*

The ER permeates the dendritic arbour and a fraction of dendritic spines (10-50% depending on region) in mammalian neurons (Spacek & Harris, 1997; Holbro and others, 2009; Ng and others, 2014; Chirillo and others, 2015; Jedlicka & Deller, 2017a). It is typically found in spines with large spine heads where it can take the form of a stacked structure known as the spine apparatus, for which the actin-binding protein synaptopodin is essential (Spacek & Harris, 1997; Cooney and others, 2002; Deller and others, 2003; Chirillo *and others*, 2015). The ER is dynamic and its localization is dependent on synaptic activity, with spine ER content being increased by NMDAR and L-type voltage-gated  $\text{Ca}^{2+}$  channel (L-VGCC) activity (Yamazaki and others, 2001; Kucharz and others, 2009; Ng and others, 2014; Chirillo and others, 2015;

Dittmer and others, 2017), and decreased by group I metabotropic glutamate receptor activity (mGluR1 and mGluR5) (Ng & Toresson, 2011).

Hippocampal dendritic spines are enriched with RyRs (Sharp and others, 1993). Early work in hippocampal pyramidal neurons, demonstrated that a significant portion of stimulation-evoked  $\text{Ca}^{2+}$  influx in the dendrites was dependent on RyRs (Obenaus and others, 1989; Alford and others, 1993). Moreover, caffeine, which triggers RyR-dependent  $\text{Ca}^{2+}$  release, was found to trigger  $\text{Ca}^{2+}$  influx in CA1 dendritic spines (Korkotian & Segal, 1998; Mainen and others, 1999). Extending these findings, Emptage and others (1999) demonstrated that  $\text{Ca}^{2+}$  influx from postsynaptic NMDARs in response to unquantal glutamate release at CA3-CA1 synapses triggered RyR activation and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) from ER stores (Figure 1). This  $\text{Ca}^{2+}$  release resembled a  $\text{Ca}^{2+}$  spark, in that it was rapid in onset and remained restricted to the spine head. In younger tissue, however, it was reported that RyR-mediated  $\text{Ca}^{2+}$  release in the spine could spread along the dendrite (Lee and others, 2016). Notably,  $\text{Ca}^{2+}$  influx from synaptic VGCCs, evoked by single back-propagating action potentials (bpAPs), failed to recruit CICR, suggesting that a local nanodomain coupling exists between synaptic NMDARs and RyRs (Emptage, 1999). However, at higher stimulation frequencies, high levels of  $\text{Ca}^{2+}$  influx from VGCCs can trigger CICR (Johanning and others, 2015).

Subsequent studies have confirmed NMDAR-induced CICR in dendritic spines, albeit with differing magnitudes (Kovalchuk and others, 2000; Korkotian & Segal, 2011; Korkotian and others, 2014; Lee *and others*, 2016), with some studies finding more robust evidence of CICR at higher stimulation frequencies (Raymond & Redman, 2006; Dittmer *and others*, 2017). By contrast, a few studies have failed to find evidence for CICR in dendritic spines altogether (Oertner and others, 2002; Sabatini and others, 2002; Holbro *and others*, 2009). The discrepant findings across the literature may be attributable to the fact that only 10-20% of CA1 spines actually contain ER, both in juvenile and adult preparations (Cooney *and others*, 2002; Holbro *and others*, 2009; Johanning *and others*, 2015). The age and recording condition may also affect the probability of observing CICR. Indeed, RyR-mediated  $\text{Ca}^{2+}$  release appears to be more robust in younger tissue (Lee *and others*, 2016), and with the use of sharp microelectrodes, which are less likely to result in the dialysis of factors supporting ER  $\text{Ca}^{2+}$  signalling (Emptage and others, 1999).

#### *Role of postsynaptic RyRs in LTP*

Synaptic CICR is likely to be important for learning and memory. Harvey and Collingridge (1992) initially demonstrated that LTP induction at CA3-CA1 synapses was inhibited by blocking  $\text{Ca}^{2+}$  release from the ER; similar findings were later reported by others (Obenaus *and others*, 1989; Harvey & Collingridge, 1992). Indeed, pharmacological inhibition of CICR, including direct inhibition of postsynaptic CA1 RyRs, impaired LTP induction at CA3-CA1 synapses (Raymond & Redman, 2002; 2006; Korkotian & Segal, 2011; Korkotian *and others*, 2014). Furthermore, genetic deletion of RyR3, which is predominately localized to postsynaptic CA1 neurons in the hippocampus, also abolished LTP induction *in vitro* and impaired spatial memory (Balschun and others, 1999) and acquisition of contextual fear conditioning *in vivo* (Kouzu and others, 2000). Suppression of learning and memory was also observed following intracerebroventricular infusions of antisense oligonucleotides against the type 2 (RyR2) or type 3 (RyR3), but not type 1 (RyR1), isoform of the RyR (Galeotti and others, 2008). By contrast, administration of RyR agonists *in vivo* improved learning retention in the Morris water maze (Adasme and others, 2011) and on a passive avoidance test (Galeotti *and others*, 2008). In line with these findings, activation of CICR *in vitro* by bath application of caffeine increased the number of surface AMPA receptors (AMPARs) in hippocampal cultures (Vlachos and others, 2009) and augmented LTP induction in hippocampal slices (Grigoryan and others, 2012; Grigoryan & Segal, 2016).

Further support for the role of CICR in LTP comes from studies of synaptopodin [see (Segal & Korkotian, 2016) and (Jedlicka & Deller, 2017b) for further reading]. Knockout of synaptopodin, which abolishes the spine apparatus and reduces expression of RYR1 (Deller *and others*, 2003; Grigoryan & Segal, 2016), impeded LTP expression in both CA1 and the dentate gyrus, and impaired spatial memory (Deller *and others*, 2003; Jedlicka and others, 2009; Zhang and others, 2013). Consistent with these findings, synaptopodin positive spines were shown to exhibit stronger  $\text{Ca}^{2+}$  signalling and to express greater amounts of LTP than synaptopodin negative spines, and in a manner dependent on RyR signalling (Vlachos *and others*, 2009; Korkotian & Segal, 2011; Korkotian *and others*, 2014). Stimulation of RyR-gated  $\text{Ca}^{2+}$  stores with caffeine also lead to selective AMPAR accumulation at synaptopodin positive spines (Vlachos *and others*, 2009), suggesting that RyR-mediated  $\text{Ca}^{2+}$  release is not only necessary, but may be sufficient for inducing postsynaptic LTP.

The importance of CICR in LTP induction is strongly dependent on the stimulation regime used to induce plasticity. Balschun and others (1999) demonstrated that LTP induction in RyR3 knockouts could be rescued, both in CA1 and dentate gyrus, by using three trains of tetanic stimulation instead of one. Similar observations were made at CA3-CA1 synapses when thapsigargin was used to inhibit CICR (Behnisch & Reymann, 1995). Moreover, Raymond and Redman (2002, 2006) found that pharmacological inhibition of RyRs impaired LTP induced by a single train of theta burst stimulation, but failed to impair LTP induced by multiple trains (4 or 8) of theta burst stimulation. Collectively, these findings suggest that RyRs predominantly facilitate LTP induction under more modest stimulation regimes, and may account as to why some studies have failed to find a role of RyRs in LTP induction (Xu, 1990; Harvey & Svoboda, 2007).

Recently, Lee and others (2016) demonstrated a role for postsynaptic RyRs in postsynaptic plasticity of CA3-CA1 synapses of young (P8-P17), but not juvenile (P20-P26), rodents. They found that in young tissue, RyRs mediated a spreading  $\text{Ca}^{2+}$  signal from active synapses, triggered by NMDAR-mediated  $\text{Ca}^{2+}$  influx, into the dendrite and neighbouring synapses. RyR-mediated  $\text{Ca}^{2+}$  influx was most likely to occur in response to Hebbian pairing of synaptic activity with postsynaptic action potentials (APs), and had the effect of reducing the threshold for LTP induction at neighbouring co-active spines. Such findings demonstrate a substantial role for synaptic CICR in shaping synaptic input during development. In keeping with this, *de novo* growth of spines, induced by glutamate release on dendritic shafts of developing cortical pyramidal neurons, was shown to be positively regulated by  $\text{Ca}^{2+}$  release from internal stores (Kwon & Sabatini, 2011).

Contrary to the majority of studies, a few groups have found that RyR activation impedes, rather than promotes, LTP induction. In particular, several studies have demonstrated that pharmacological inhibition of postsynaptic RyRs (Wang and others, 1996; Nishiyama and others, 2000), or genetic deletion of RyR3 (Futatsugi and others, 1999), augments LTP induction in dentate gyrus and CA1, with a corresponding improvement in spatial memory. In these studies, the improved LTP induction was shown to depend on L-VGCC, as opposed to NMDAR, activity (Futatsugi *and others*, 1999), and is therefore reminiscent of the L-VGCC-dependent, but NMDAR-independent, form of presynaptic plasticity that is now known to be expressed at CA3-CA1 synapses (Blundon & Zakharenko, 2008; Padamsey & Emptage, 2014;

Padamsey *and others*, 2017c). It was also recently shown that postsynaptic CICR inhibits L-VGCC activity in a manner dependent on STIM1, the ER  $\text{Ca}^{2+}$  sensor responsible for activating store-operated  $\text{Ca}^{2+}$  channels (Dittmer *and others*, 2017). Thus, it is possible that postsynaptic RyR activation promotes the induction of postsynaptic LTP by augmenting NMDAR-associated  $\text{Ca}^{2+}$  influx (Emptage *and others*, 1999), but impairs the induction of presynaptic LTP by inhibiting L-VGCC activity. Notably, the type of stimulation used to induce plasticity determines the locus of LTP expression. In particular, whereas LTP induction is almost always accompanied by a NMDAR-dependent, postsynaptic component of expression, an additional L-VGCC-dependent, presynaptic component of expression is only observed with induction protocols leading to strong postsynaptic depolarization (e.g. high frequency or high intensity stimulation, or low frequency pairing of presynaptic stimuli with complex/dendritic spikes) (Padamsey & Emptage, 2014; Padamsey *and others*, 2017c). Consequently, the net effect of RyRs on LTP, whether facilitatory or inhibitory, will very likely depend on the stimulation parameters used to induce plasticity.

#### *Role of postsynaptic RyRs in LTD*

Postsynaptic RyRs have also been implicated in LTD induction. Indeed, several groups demonstrated that pharmacological inhibition of postsynaptic RyRs, or genetic knockout of RyR3, inhibited LTD induction at CA3-CA1 synapses by low frequency (1-5 Hz) afferent stimulation (Wang *and others*, 1996; Futatsugi *and others*, 1999). Depotentiation at perforant path synapses in the dentate gyrus was also impaired (O'Mara *and others*, 1995; Wang *and others*, 1997; Balschun *and others*, 1999); though, depotentiation at CA3-CA1 synapses was unaffected (Reyes & Stanton, 1996). Inhibition of postsynaptic RyRs also inhibited LTD induced by a spike-timing dependent plasticity protocol (STDP) at CA3-CA1 synapses, in which presynaptic stimuli were anti-causally paired with postsynaptic APs (Nishiyama *and others*, 2000). In a subsequent study, when glutamate uncaging was used instead of afferent stimulation during STDP-LTD induction, LTD persisted in the absence of store  $\text{Ca}^{2+}$  release, albeit at a reduced amplitude (Hayama *and others*, 2013). It is possible that glutamate uncaging, which would likely activate synapses with greater potency and reliability than afferent stimulation, overcomes the absolute necessity for RyR-mediated  $\text{Ca}^{2+}$  release in LTD induction. Thus, as with LTP induction, ER  $\text{Ca}^{2+}$  may only be essential in LTD induction under moderate stimulation regimes. Moreover, as with LTP induction, postsynaptic RyRs also

appear to namely promote forms of LTD that are expressed postsynaptically rather than presynaptically (Reyes & Stanton, 1996; Reyes-Harde and others, 1999; Unni and others, 2004) [see *Role of presynaptic RyRs in long-term presynaptic plasticity*].

#### *Postsynaptic IP<sub>3</sub>Rs mediate Ca<sup>2+</sup> release in dendrites and spines*

In hippocampal pyramidal cells, IP<sub>3</sub>Rs are particularly enriched in dendritic branches, though can be present in spines (Sharp *and others*, 1993). As with RyRs, IP<sub>3</sub>Rs can mediate Ca<sup>2+</sup> release at single spines in response to glutamate release (Figure 2A). Direct evidence was provided by Holbro and others (2009), who demonstrated that either glutamate uncaging or focal synaptic stimulation, in addition to eliciting a rapid NMDAR-mediated Ca<sup>2+</sup> influx, could elicit a delayed and prolonged IP<sub>3</sub>R-dependent Ca<sup>2+</sup> transient in single CA1 spines. This signal depended on the activation of G<sub>q</sub>-coupled Group I mGluRs (mGluR1 and mGluR5). Importantly, IP<sub>3</sub>R-mediated Ca<sup>2+</sup> transients only occurred in spines containing ER and remained restricted to the spine head.

Other IP<sub>3</sub>R-dependent Ca<sup>2+</sup> signalling motifs exist. Several groups have demonstrated that high frequency stimulation of spatially clustered synapses can trigger IP<sub>3</sub>R-mediated Ca<sup>2+</sup> waves (Figure 2B). These have been shown to bidirectionally propagate along the dendrite, and can, in some cases invade the soma and nucleus (Wiegert & Bading, 2011). This phenomenon has been reported for principal neurons in hippocampus, cortex, and the basolateral amygdala, with the spatial extent of Ca<sup>2+</sup> spread being dependent on the intensity and frequency of stimulation (Nakamura and others, 1999; Nakamura and others, 2002; Zhou & Ross, 2002; Hagenston and others, 2007). These Ca<sup>2+</sup> waves are inhibited by antagonists to IP<sub>3</sub>Rs, but not RyRs, and depend on the activation of Group 1 mGluRs (Jaffe & Brown, 1994; Nakamura *and others*, 1999; Kapur and others, 2001; Hagenston *and others*, 2007; Power & Sah, 2007; 2014); though, can also be mediated by activation of cholinergic and serotonergic G<sub>q</sub>-coupled receptors (Nakamura and others, 2000; Nakamura *and others*, 2002; Power & Sah, 2002; Larkum and others, 2003). In general, IP<sub>3</sub>R-mediated waves are predominantly detected in the proximal apical trunk, as opposed to the distal apical and basal dendrites (Nakamura *and others*, 1999; Nakamura *and others*, 2002; Power & Sah, 2007). However, there is evidence to suggest that these waves are initiated in the first few microns of oblique dendrites, which receive the majority of synaptic contacts, and are subsequently summated and amplified at the main apical branch (Larkum *and others*, 2003).

Since IP<sub>3</sub>R activation requires both IP<sub>3</sub> and Ca<sup>2+</sup> (Taylor & Tovey, 2010), Ca<sup>2+</sup> influx through NMDARs or VGCCs can act synergistically with the activation of G<sub>q</sub>-coupled receptors to trigger Ca<sup>2+</sup> waves (Figure 2B). For example, IP<sub>3</sub>R-mediated Ca<sup>2+</sup> waves can be triggered by APs, delivered in the presence of G<sub>q</sub>-coupled receptor agonists (Nakamura *and others*, 1999; Nakamura *and others*, 2000; Yamamoto and others, 2000; Nakamura *and others*, 2002; Larkum *and others*, 2003; Stutzmann and others, 2003). Similarly, the frequency or intensity of synaptic stimulation required to trigger IP<sub>3</sub>R-mediated Ca<sup>2+</sup> waves can be reduced if stimulation is paired with a brief burst of APs, which promote Ca<sup>2+</sup> influx via VGCCs (Nakamura *and others*, 1999; Kapur *and others*, 2001; Power & Sah, 2007). Ca<sup>2+</sup> influx via NMDARs, can similarly reduce the threshold for activating Ca<sup>2+</sup> waves (Nakamura *and others*, 2002). The facilitatory effect of NMDAR- or VGCC-mediated Ca<sup>2+</sup> influx can also occur over a time scale of minutes by promoting Ca<sup>2+</sup> loading of IP<sub>3</sub>R-gated stores during synaptic activity and neuronal spiking (Rae and others, 2000; Stutzmann *and others*, 2003; Hagenston *and others*, 2007). Thus, while activation of isolated synapses can support local IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release (Holbro *and others*, 2009), cooperative activity across a population of synapses, both in time and space, can lead to higher levels of IP<sub>3</sub> production and NMDAR/VGCC-dependent Ca<sup>2+</sup> influx, which triggers Ca<sup>2+</sup> waves that propagate along the dendrite.

#### *Role of postsynaptic IP<sub>3</sub>Rs in postsynaptic LTD*

Holbro and others (2009) demonstrated that localized IP<sub>3</sub>R-mediated Ca<sup>2+</sup> influx in single spines, stimulated with glutamate uncaging at low frequencies, lead to postsynaptic LTD and spine shrinkage in CA1 neurons. LTD was mGluR-dependent, but NMDAR-independent, and only observed at synapses containing ER, most of which were also found to contain synaptopodin. By contrast, Oh and others (2012), found that low frequency glutamate uncaging produced NMDAR-dependent LTD at single spines. However, they also found that LTD induction at larger spines, which are more likely to contain ER, was additionally dependent on Group 1 mGluR and IP<sub>3</sub>R signalling, whereas LTD induction at smaller spines only required NMDAR activation (Oh and others, 2012). Thus, despite their differences, findings from both studies suggest that spines containing IP<sub>3</sub>R-gated stores require IP<sub>3</sub>R-mediated Ca<sup>2+</sup> signalling for homosynaptic LTD, whereas those lacking ER appear to rely only on NMDAR activity. Notably, genetic knockout of IP<sub>3</sub>R1, the major neuronal form of IP<sub>3</sub>Rs and particularly enriched in CA1, failed to block LTD induction at CA3-CA1 synapses by low



frequency electrical stimulation (Fujii and others, 2000; Nagase and others, 2003). A subsequent study by the same group, however, did demonstrate that pharmacological inhibition of IP<sub>3</sub>Rs inhibited LTD induction, and suggested that the effects of IP<sub>3</sub>R1 KOs were potentially confounded by compensatory effects during development, or, alternatively, altered synaptic function in the absence of IP<sub>3</sub>Rs (Taufiq and others, 2005).

In addition to homosynaptic LTD, synapses can undergo heterosynaptic LTD postsynaptically, which is traditionally thought to result from a spread of Ca<sup>2+</sup> from active synapses. Rather than being triggered by low intensity, low frequency stimulation ( $\leq 1$  Hz), which is most likely to be associated with synaptically localized IP<sub>3</sub>R-mediated Ca<sup>2+</sup> influx (Holbro *and others*, 2009), heterosynaptic LTD is typically triggered by stronger stimulation regimes, which are more likely to be associated with postsynaptic IP<sub>3</sub>R-mediated Ca<sup>2+</sup> waves (Nakamura *and others*, 1999). These stimulation regimes either involve either higher stimulation frequencies (1-50Hz) (Lynch and others, 1977; Dunwiddie & Lynch, 1978; Nagase *and others*, 2003), pairing of low frequency synaptic activity with APs (Nishiyama *and others*, 2000; Hayama *and others*, 2013), or activation of multiple synapses in close proximity (Oh and others, 2015). In addition to inducing heterosynaptic LTD, these protocols induce homosynaptic LTD (Dunwiddie & Lynch, 1978; Nishiyama *and others*, 2000; Nagase *and others*, 2003; Hayama *and others*, 2013), or, if synaptic activity is sufficiently strong, homosynaptic LTP (Lynch *and others*, 1977; Dunwiddie & Lynch, 1978; Power & Sah, 2014; Oh *and others*, 2015). In keeping with the role of IP<sub>3</sub>Rs in LTD, a number of studies find that genetic deletion (Nagase *and others*, 2003) of IP<sub>3</sub>R1, or pharmacological inhibition of IP<sub>3</sub>Rs (Oh *and others*, 2015), including specific blockade of postsynaptic IP<sub>3</sub>Rs (Nishiyama *and others*, 2000) abolish heterosynaptic LTD, and accompanying homosynaptic LTD when it is present (Nishiyama *and others*, 2000).

Not all forms of heterosynaptic LTD require IP<sub>3</sub>R signalling. Hayama and others (2013) paired glutamate uncaging at a single synapse with an AP in an anticausal manner. Pairings were made at a low frequency, and in the presence of GABAergic signalling. This protocol resulted in the induction of homosynaptic LTD that spread to neighbouring synapses, even when ER Ca<sup>2+</sup> signalling was inhibited. The authors demonstrated that local uncaging of glutamate (in the presence of either GABA or a GABA<sub>A</sub>R agonist) influenced the spatial signature of voltage-dependent Ca<sup>2+</sup> influx at and around the target synapse, independent of store release, and that this modulation was sufficient to trigger both homo- and heterosynaptic LTD.

In addition to regulating LTD, IP<sub>3</sub>Rs have been reported to mediate other related forms of plasticity, including depotentiation and LTP suppression, which is a metaplastic effect where low frequency stimulation, delivered prior to tetanic stimulation, prevents the subsequent induction of LTP (Fujii *and others*, 2000; Yamazaki and others, 2012; Fujii and others, 2016).

#### *Role of postsynaptic IP<sub>3</sub>Rs in LTP*

Postsynaptic IP<sub>3</sub>Rs have also been implicated in the induction of LTP. Raymond and others (2002,2006) demonstrated that inhibition of IP<sub>3</sub>Rs prevented LTP induction induced by four, but not to one or eight, trains of theta burst stimulation. Thus, IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release appears to be important for LTP induced with moderate stimulation. Weak stimulation may not be sufficient to recruit IP<sub>3</sub>R-mediated Ca<sup>2+</sup> influx, whereas strong stimulation may recruit sufficient Ca<sup>2+</sup> influx from other sources, such as NMDARs or VGCCs, for LTP induction without the need for additional store release. Such findings are consistent with the observation that Group 1 mGluRs are important only for LTP induced by modest induction protocols (Wilsch and others, 1998); their involvement in synaptic plasticity, therefore, is not consistently reported across studies. During theta burst stimulation, IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release has been reported to occur mainly in the dendritic shaft (as opposed to spine heads) (Raymond & Redman, 2006), where it is postulated to trigger the protein synthesis required for LTP (Raymond and others, 2000).

IP<sub>3</sub>Rs can also impede LTP induction. Several groups, have reported that either genetic knockout of IP<sub>3</sub>R1 (Fujii *and others*, 2000; Nishiyama *and others*, 2000; Nagase *and others*, 2003; Yoshioka and others, 2010) or pharmacological inhibition of IP<sub>3</sub>Rs (Taufiq *and others*, 2005), including blockade of postsynaptic IP<sub>3</sub>Rs (Nishiyama *and others*, 2000; Nagase *and others*, 2003), augments LTP induced by tetanic stimulation or STDP protocols. It currently remains unclear as to the exact conditions under which IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release acts to impede or promote LTP induction.

#### *Presynaptic RyRs mediate Ca<sup>2+</sup> release in axonal boutons*

In addition to postsynaptic compartments, the ER and its associated receptors can be found throughout axons and boutons, where the expression of RyRs, rather than that of IP<sub>3</sub>Rs dominate (Sharp *and others*, 1993). Emptage and others (2001) provided direct evidence that single APs triggered highly localized, RyR-dependent CICR at CA3 terminals, likely triggered by

Ca<sup>2+</sup> influx via VGCCs (Figure 3). These events, resembling Ca<sup>2+</sup> sparks, were also found to occur spontaneously at presynaptic terminals in the absence of evoked activity, and contributed to spontaneous transmitter release (Emptage and others, 2001). Subsequently, Unni and others (2004) found that low frequency (5Hz) stimulation resulted in a slow accumulation of Ca<sup>2+</sup> within boutons that was dependent on presynaptic RyRs. In contrast, de Juan-Sanz (2017) found that high frequency (20 Hz) and prolonged (1s) stimulus trains produced net loading, rather than depletion, of ER Ca<sup>2+</sup> stores, suggesting that under these more intense stimulation conditions the dominant function of the ER is to sequester cytosolic Ca<sup>2+</sup> within the bouton.

#### *Role of presynaptic RyRs in short-term presynaptic plasticity*

Despite single APs eliciting CICR, Emptage and others (2001) found no effect of CICR on basal release probability at hippocampal synapses ( $P_r$ ); though modest, CICR-dependent elevations in  $P_r$  have been reported by others (Cabezas & Buno, 2006). They did, however, find that inhibition of RyRs greatly impaired paired pulse facilitation, suggesting that CICR supports short-term presynaptic plasticity. The effect of CICR in short-term facilitation is predominantly observed at moderate (10-20 Hz), but not low (< 5 Hz), stimulation frequencies (Emptage *and others*, 2001; Cabezas & Buno, 2006; Zhang and others, 2009). Notably, at postsynaptically silent synapses, inhibition of RyRs had no impact on transmitter release, suggesting that presynaptic CICR is dependent on synapse state (Cabezas & Buno, 2006). Collectively, the findings from these studies provide strong evidence for the existence of CICR across CA3-CA1 synapses, and suggest that presynaptic RyRs can regulate transmitter release in a manner dependent on stimulation frequency. One study did fail to find an effect of CICR inhibition on evoked release (Carter and others, 2002). This failure may be attributable to the experiment not being conducted at a physiological temperature, which is known to impact CICR (de Juan-Sanz and others, 2017).

#### *Role of presynaptic RyRs in long-term presynaptic plasticity*

Reyes and Stanton (1996) initially demonstrated that a form of NMDAR-dependent LTD, induced by low frequency stimulation (1 Hz) at CA3-CA1 synapses, selectively depended on the activation of pre-, but not post-, synaptic RyRs. These findings were later confirmed by Unni and others (2004), who additionally demonstrated this form of LTD was expressed

presynaptically, and required RyR-dependent accumulation of  $\text{Ca}^{2+}$  in the bouton during stimulation. However, whereas Reyes and Stanton (1996) found an additional requirement of postsynaptic  $\text{IP}_3\text{Rs}$  in presynaptic LTD induction, no such requirement was reported by Unni and others (2004); although, it is possible that the higher stimulation frequency (5 Hz) used by Unni and others (2004) to induce LTD bypassed the need for postsynaptic  $\text{IP}_3\text{R}$  involvement. Both groups did however demonstrate that LTD could be abolished by bath application of NMDAR antagonists; although it is not clear whether this effect was mediated by pre- or postsynaptic NMDARs. Recent findings have demonstrated that pre-, rather than post-, synaptic NMDARs are essential for the induction of presynaptic LTD at CA3-CA1 synapses by low frequency (5 Hz) stimulation (Padamsey *and others*, 2017c). Such findings hint at an intriguing possibility: that  $\text{Ca}^{2+}$  influx through presynaptic NMDARs triggers RyR-mediated  $\text{Ca}^{2+}$  release, resulting in the induction of LTD, possibly via the activation of presynaptic CAMKII (Stanton & Gage, 1996). This would suggest that the functional coupling of NMDARs and RyRs is preserved at both pre- and postsynaptic loci. In addition to LTD, presynaptic RyRs have been shown to mediate the induction of presynaptic LTP induced either by caffeine application (Martin & Buno, 2003), or by stimulation of presynaptic,  $\text{Ca}^{2+}$  permeable cholinergic receptors with nicotine (Le Magueresse & Cherubini, 2007).

## **Mitochondria**

Mitochondria play an important role in shaping neuronal  $\text{Ca}^{2+}$  signalling. They have a potent ability to sequester  $\text{Ca}^{2+}$  that arises from their highly hyperpolarized resting membrane potential (-180 mV). This ensures rapid transport of cytosolic  $\text{Ca}^{2+}$  down its electrochemical gradient, into the organelle, likely via the action of a  $\text{Ca}^{2+}$  uniporter (Peng and others, 1998; Contreras and others, 2010) [but see (Jiang and others, 2009)]. Indeed, intramitochondrial  $\text{Ca}^{2+}$  levels can reach hundreds of micromolar even in response to a modest, micromolar increase in cytosolic  $\text{Ca}^{2+}$  (Contreras *and others*, 2010).  $\text{Ca}^{2+}$  release from the mitochondria can also occur either by  $\text{Ca}^{2+}/\text{Na}^+$  exchange (Chiesi and others, 1988), or via the opening of the mitochondrial permeability transition pore, which is triggered by an increase in intramitochondrial  $\text{Ca}^{2+}$ , and enables the release of small molecules into the cytosol (Rasola & Bernardi, 2007). Sigma-1 receptors, found on mitochondrion-associated ER membrane (MAM), can also impact mitochondrial  $\text{Ca}^{2+}$  signalling; their regulation of synaptic

transmission and plasticity is beyond the scope of this review, but see (Kourrich and others, 2012) for further details.

#### *Role of presynaptic mitochondria in presynaptic $\text{Ca}^{2+}$ signalling and plasticity*

Mitochondria are found throughout the axon, where they predominantly localize to presynaptic terminals. Approximately 50% of all boutons are in the proximity ( $\leq 0.5 \mu\text{m}$ ) of a mitochondrion (Kang and others, 2008). As common to other  $\text{Ca}^{2+}$  stores, mitochondria are not static, but show complex patterns of mobility, likely concentrating in regions of high metabolic demand (Morris & Hollenbeck, 1993; Kang *and others*, 2008; Lin & Sheng, 2015).

Tang and Zucker (1997) established the importance of presynaptic mitochondria in post-tetanic potentiation at the crayfish neuromuscular junction (NMJ) (Figure 4). They found that the mitochondria sequestered cytosolic  $\text{Ca}^{2+}$  during a prolonged train (7-10 minutes) of high frequency (20-33 Hz) stimulation, and subsequently released the  $\text{Ca}^{2+}$ , over tens of minutes. Concurrent with these changes, transmitter release was reduced during stimulation but enhanced after. Similar effects have been shown for brief (5 s) trains of high frequency stimulation (100 Hz) at the rodent NMJ, but critically only at physiological temperatures; cooler temperatures compromise mitochondrial  $\text{Ca}^{2+}$  regulation (David & Barrett, 2000). At the mammalian Calyx of Held, mitochondria also buffered  $\text{Ca}^{2+}$  release during high frequency stimulation (100 Hz, 200 ms), but this was not accompanied by a long-lasting increase in either cytosolic  $\text{Ca}^{2+}$  level or transmitter release following the cessation of stimulation (Billups & Forsythe, 2002). The lack of long-lasting increases may reflect the much shorter duration of stimulation used in this study (200 ms), as compared to the studies at the NMJ (5 s) (David & Barrett, 2000), which likely results in greater  $\text{Ca}^{2+}$  loading of the mitochondria. Thus, the induction of mitochondrial-dependent post-tetanic potentiation is likely to depend on stimulation conditions.

Although stimulation may not always trigger mitochondrial-dependent post-tetanic potentiation, inhibition of mitochondrial  $\text{Ca}^{2+}$  signalling during high frequency stimulation appears to robustly trigger post-tetanic depression. Such depression has been reported both at the Calyx of Held (Billups & Forsythe, 2002) and the rodent NMJ during high (100 Hz), but not low (1 Hz), frequency stimulation (David & Barrett, 2003). Post-tetanic depression is thought to occur from the elevation of cytosolic  $\text{Ca}^{2+}$  that occurs during axonal stimulation

following inhibition of mitochondrial  $\text{Ca}^{2+}$  uptake. This elevated  $\text{Ca}^{2+}$  has been shown to markedly increase asynchronous release during stimulation, resulting in vesicle depletion that lasts for seconds after stimulation (David & Barrett, 2003).  $\text{Ca}^{2+}$  dependent inhibition of endocytosis may also contribute to vesicle depletion (Billups & Forsythe, 2002). Thus, mitochondrial  $\text{Ca}^{2+}$  uptake during high frequency activity is essential for suppressing post-tetanic depression, at least at the mammalian Calyx of Held and NMJ.

Despite these findings, a number of groups have failed to find a role for mitochondria in regulating transmitter release at hippocampal synapses. Indeed, several studies have found that, vesicle fusion and  $\text{Ca}^{2+}$  influx (evoked by 10 Hz stimulation) at hippocampal boutons is comparable, even at physiological temperatures, regardless of whether or not they contain mitochondria (Waters & Smith, 2003; Sun and others, 2013). For repeated trains of 10 Hz stimulation, sustained release is only seen at boutons containing mitochondria, though this effect is likely mediated by mitochondrial production of ATP rather than  $\text{Ca}^{2+}$  signalling (Sun and others, 2013). Knockdown of the mitochondrial  $\text{Ca}^{2+}$  uniporter similarly has no effect on vesicle release or cytosolic  $\text{Ca}^{2+}$  levels during 10 Hz stimulation, nor does pharmacological inhibition of mitochondrial  $\text{Ca}^{2+}$  uptake in hippocampal synaptosomes (Ivannikov and others, 2013). Curiously, direct imaging of mitochondrial  $\text{Ca}^{2+}$  reveals that mitochondria do buffer elevations in cytosolic  $\text{Ca}^{2+}$  at hippocampal boutons, even in response to single APs, and in a manner dependent on the mitochondrial  $\text{Ca}^{2+}$  uniporter (Gazit and others, 2016). Nonetheless, it would appear at first sight that  $\text{Ca}^{2+}$  sequestration has no impact on basal transmitter release or presynaptic short-term plasticity at these synapses. Perhaps of relevance is that the effects of mitochondrial  $\text{Ca}^{2+}$  signalling on presynaptic function are frequency dependent, and therefore may not to be apparent at the lower frequency (10 Hz) stimulation regimes normally used in hippocampal studies, but present at the higher frequencies (100-200 Hz) used in studies of the NMJ and the Calyx of Held. Indeed, at least at the NMJ, the effects of mitochondrial  $\text{Ca}^{2+}$  signalling on vesicle release are only observed at high (100 Hz), and not low (1 Hz), stimulation frequencies (David & Barrett, 2003). Given that hippocampal pyramidal neurons can burst fire at approximately 100-300 Hz *in vivo* (Kowalski and others, 2016), it would be valuable to assess whether mitochondria impact vesicle release at higher stimulation frequencies.

Despite the lack of evidence that mitochondrial  $\text{Ca}^{2+}$  signalling regulates vesicle exocytosis at hippocampal synapses, mitochondria may regulate presynaptic function by other means. Marland and others (2016), for example, found that vesicle endocytosis during 10 Hz stimulation was accelerated by knockdown of the mitochondrial  $\text{Ca}^{2+}$  uniporter, and slowed by overexpression of the uniporter, suggesting that mitochondrial  $\text{Ca}^{2+}$  sequestration impeded vesicle retrieval (Marland and others, 2016). Notably, neither of these manipulations made any obvious impact on cytosolic  $\text{Ca}^{2+}$  levels, as assessed by the genetically encoded  $\text{Ca}^{2+}$  sensors, and so the data were interpreted as affecting nanodomain  $\text{Ca}^{2+}$  signalling in the vicinity of sites of endocytosis (Marland *and others*, 2016). Moreover, Gazit and others (2016) demonstrated that basal activity of presynaptic insulin growth factor receptors (IGFRs) promoted mitochondrial  $\text{Ca}^{2+}$  uptake, resulting in a decrease in resting bouton  $\text{Ca}^{2+}$  levels, and a concurrent decrease in spontaneous transmitter release. IGFR activity also promoted mitochondrial ATP production, which was essential for maintaining evoked  $\text{Ca}^{2+}$  influx and evoked vesicle release. Furthermore, genetic disruption or pharmacological inhibition of the mitochondrial permeability transition pore, which can alter cytosolic  $\text{Ca}^{2+}$  (Rasola & Bernardi, 2007), was reported to increase basal  $P_r$ , as assessed by a decrease in paired pulse facilitation. Such changes were associated with impairments in associative and spatial learning, as well as in hippocampal LTP induction (Weeber and others, 2002; Levy and others, 2003). Finally, at perforant path synapses, LTP induction by high frequency stimulation (100 Hz) was reported to induce long-lasting increases in mitochondrial  $\text{Ca}^{2+}$  uptake (Stanton & Schanne, 1986); although the consequence of this increase has yet to be elucidated, such long-term changes in mitochondrial  $\text{Ca}^{2+}$  may support the long-term changes in presynaptic function that are associated with perforant path LTP (Bliss and others, 1990).

#### *Role of postsynaptic mitochondria in postsynaptic $\text{Ca}^{2+}$ signalling and plasticity*

Mitochondria are also found throughout dendrites, including some dendritic spines (Cameron and others, 1991; Popov and others, 2005), though not those of CA3-CA1 synapses (Spacek & Harris, 1997). As in the axon, they redistribute in an activity-dependent manner to regions of high metabolic demand (Li and others, 2004).

Mitochondria play a critical role in postsynaptic plasticity. In particular, mitochondrial activation of caspase-3 has been shown to be essential for mediating AMPAR internalization

during the induction of NMDAR-, but not mGluR-, dependent LTD at CA3-CA1 synapses during low frequency stimulation (1 Hz) (Li and others, 2010; Jiao & Li, 2011). Caspase-3 can also induce shrinkage of postsynaptic spines and retraction of dendrites (Erturk and others, 2014). Its activation requires the recruitment of the BCL-2 family protein BAD by NMDAR-dependent,  $\text{Ca}^{2+}$ -sensitive phosphatases (PP1, PP2A, PP2B/calcineurin). BAD relieves inhibition of the pore-forming protein BAX, leading to mitochondrial permeabilization and the release of cytochrome c, which in turn, triggers the activation of caspase-3 through the actions of the apoptosome (Li *and others*, 2010; Jiao & Li, 2011). Although traditionally thought to be selectively associated with apoptosis, this pathway is recruited, albeit to a modest extent, with physiological levels of stimulation (Jiao & Li, 2011).

The specific role of the mitochondrial  $\text{Ca}^{2+}$  signalling in the process of cytochrome c/caspase-3 recruitment has not been directly assessed in neurons. However, in other cell types, it is established that BAX triggers the release of cytochrome c through its actions on the mitochondrial permeability transition pore, and in a manner dependent on mitochondrial  $\text{Ca}^{2+}$  (Narita and others, 1998; Pastorino and others, 1999). It is therefore possible that mitochondrial sequestration of  $\text{Ca}^{2+}$  during NMDAR-dependent stimulation, previously reported in neurons (Peng *and others*, 1998; Kannurpatti and others, 2000), triggers cytochrome c release. Moreover, cytochrome c release can potentiate its own release by eliciting  $\text{Ca}^{2+}$  release from  $\text{IP}_3\text{R}$ -gated stores, leading to further mitochondrial  $\text{Ca}^{2+}$  sequestration, and therefore further cytochrome c release (Boehning and others, 2003; Hanson and others, 2004). Consistent with this notion,  $\text{IP}_3\text{R}$ -mediated  $\text{Ca}^{2+}$  release was found to be essential for caspase-3 recruitment during the induction of NMDAR-dependent LTD (Li *and others*, 2010).

### **Acidic $\text{Ca}^{2+}$ stores**

Acidic  $\text{Ca}^{2+}$  stores, such as lysosomes, were once thought of as exclusively degradative organelles. However, they are now well established as  $\text{Ca}^{2+}$  stores distinct from the ER and mitochondria (Galione and others, 2010; Galione, 2014). Acidic  $\text{Ca}^{2+}$  stores have been found to mediate physiological functions in a number of cell types, including contractility of cardiac myocytes, insulin release in pancreatic beta cells, and differentiation of osteoclasts (Galione *and others*, 2010). Acidic stores sequester  $\text{Ca}^{2+}$  using a  $\text{H}^+$  gradient, generated by a vacuolar  $\text{H}^+$ -ATPase (V-ATPase), and a  $\text{Ca}^{2+}/\text{H}^+$  exchange mechanism (Morgan and others, 2011) [but



see (Garritty and others, 2016)].  $\text{Ca}^{2+}$  release from these stores requires the action of the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP), likely synthesized by ADP-ribosyl cyclases in response to cell-specific physiological stimuli (Cosker and others, 2010; Galione *and others*, 2010; Galione, 2014). The exact mechanism by which NAADP drives  $\text{Ca}^{2+}$  release from the acidic stores is unclear, but there is evidence to suggest that NAADP may act on two-pore channels (TPCs), possibly via an NAADP-associated binding protein (Morgan & Galione, 2013; Morgan and others, 2015); though, other putative NAADP binding channels, such as the transient receptor potential cation channels of the mucolipin 1 family (TRPML1) have also been proposed (Zhang & Li, 2007; Guse, 2012).

#### *Role of postsynaptic acidic stores in postsynaptic $\text{Ca}^{2+}$ release and plasticity*

Recent studies have started to elucidate the role of lysosomal  $\text{Ca}^{2+}$  signalling in neurons. Lysosomes are found throughout the dendritic arbour, including a fraction of dendritic spines (Padamsey and others, 2017a). As with the ER and mitochondria, the localization of these organelles is dynamic and activity-dependent, with glutamatergic signalling recruiting lysosomes to the base of spine heads (Goo and others, 2017). Padamsey and others (2017) reported that bpAPs in hippocampal pyramidal neurons elicited  $\text{Ca}^{2+}$  release from dendritic lysosomes (Figure 5). Remarkably, this release triggered the fusion of the lysosome itself with the plasma membrane, resulting in the exocytosis of its luminal contents. Although many lysosomal enzymes are inactive in the pH neutral environment of the extracellular matrix (ECM), some, such as Cathepsin B, retain their activity (Mort and others, 1984; Linebaugh and others, 1999). Padamsey and others (2017) demonstrated that Cathepsin B, when released, recruited extracellular MMP-9 activity by cleaving its endogenous inhibitor, TIMP-1 (Kostoulas and others, 2000; Murphy & Lynch, 2012). MMP-9 is well known as an ECM remodelling enzyme, and is essential for the maintenance of the functional and structural changes associated with LTP (Wang and others, 2008; Wlodarczyk and others, 2011; Dziembowska & Wlodarczyk, 2012). Consistent with this, pharmacological inhibition of lysosomal fusion or Cathepsin B activity, both of which impaired MMP9 activity, prevented long-term maintenance of dendritic spine enlargements induced by Hebbian activity. Moreover, chronic inhibition of lysosomal function or  $\text{Ca}^{2+}$  signalling, which is implicated in lipid storage disorders, altered spine morphology and reduced spine density (Goo *and others*, 2017;

Padamsey and others, 2017b). Thus, activity-dependent  $\text{Ca}^{2+}$  release from dendritic lysosomes plays a critical role in regulating postsynaptic plasticity and synaptic morphology.

In addition to bpAPs, glutamate release can also trigger lysosomal  $\text{Ca}^{2+}$  release in neurons and glia, likely by triggering NAADP synthesis (Pandey and others, 2009); though, the functional effects of such release have not been investigated. Moreover, acidic store  $\text{Ca}^{2+}$  signalling has also been found to induce membrane depolarization in medullary (Brailoiu and others, 2009) and hippocampal neurons (unpublished data), as well to regulate the surface expression of N-type voltage-gated  $\text{Ca}^{2+}$  channels (Hui and others, 2015) and neurite outgrowth in developing neurons (Arantes & Andrews, 2006). These functions may provide additional mechanisms by which acidic stores can influence synaptic and network activity.

#### *Presynaptic acidic stores mediate presynaptic $\text{Ca}^{2+}$ release*

Lysosomes are also found along axons and in axonal terminals. In CA3 neurons, McGuinness and others (2007) found that lysosomes generated increases in bouton  $\text{Ca}^{2+}$  in response to single APs. This  $\text{Ca}^{2+}$  release, unlike in dendrites, was additionally dependent on ER  $\text{Ca}^{2+}$  stores (Figure 3). Such functional coupling between lysosomes and ER have been documented in other cell types, where typically, lysosomes release a nanodomain plume of  $\text{Ca}^{2+}$ , which subsequently is amplified via RyR or  $\text{IP}_3\text{R}$  activation (Petersen & Cancela, 1999; Patel and others, 2001). Notably, activity-dependent lysosomal  $\text{Ca}^{2+}$  release in both axons and dendrites was found to depend on  $\text{Ca}^{2+}$  influx from VGCCs (McGuinness and others, 2007; Padamsey and others, 2017a) (Figure 5). The mechanism of coupling between VGCCs and lysosomes is not understood. However, that TPCs are themselves regulated by  $\text{Ca}^{2+}$  (Pitt and others, 2010; Pitt and others, 2014), suggests that VGCC-mediated  $\text{Ca}^{2+}$  influx may trigger lysosomal  $\text{Ca}^{2+}$  influx by acting on these channels. Such coupling would conveniently endow lysosomes with voltage sensitivity, allowing them to be regulated by neuronal activity.

The role of presynaptic lysosomes in presynaptic function has not been extensively studied. However, it is known that  $\text{Ca}^{2+}$  release from these stores can drive spontaneous transmitter release at CA3 terminals, without an obvious impact on transmitter release evoked by single APs (McGuinness and others, 2007). These stores also appear to affect transmitter release at non-mammalian synapses [for a review see (Bezin and others, 2006)].

## **Conclusions**

Here, we have discussed the  $\text{Ca}^{2+}$  signalling properties of the ER, mitochondria, and acidic  $\text{Ca}^{2+}$  stores, and the role each plays in synaptic plasticity (Table 1). Despite the incredible diversity of signalling across these stores, some common themes have emerged. 1) Stores are dynamic and relocate in an activity-dependent manner. 2) Presynaptic stores largely influence presynaptic plasticity, and postsynaptic stores largely influence postsynaptic plasticity. 3) The extent to which a store is involved in regulating synaptic function and plasticity critically depends upon the pattern of neuronal activity, including the intensity and frequency of stimulation (Table 1). Despite the wealth of published literature, further research is required to better elucidate the role of  $\text{Ca}^{2+}$  stores in synaptic function in plasticity, especially in the case of acidic stores and mitochondria, for which there is much still to learn.

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### Figure captions:

**Figure 1.** Postsynaptic RyR signalling. NMDAR-mediated  $\text{Ca}^{2+}$  influx triggers CICR from spine RyR stores. Back-propagating action potentials (bpAPs) can also trigger CICR, either by augmenting NMDAR-mediated  $\text{Ca}^{2+}$  influx, or by triggering  $\text{Ca}^{2+}$  influx from VGCCs when present if they occur at high frequency. CICR is generally restricted to the spine head, though can spread into the dendrites in younger tissue, and can result in either LTP or LTD, depending on the pattern of synaptic activity (see Table 1).

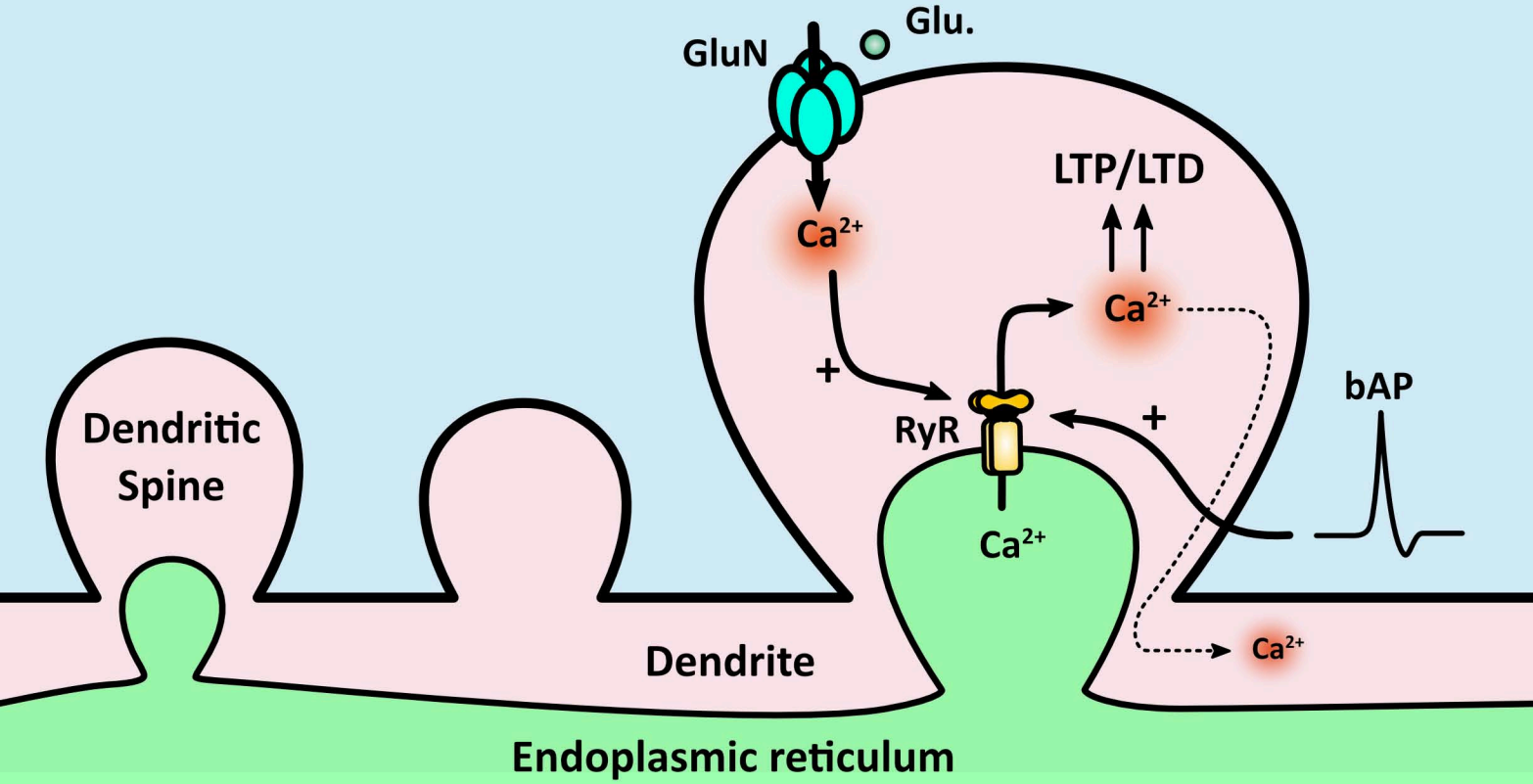
**Figure 2.** Postsynaptic  $\text{IP}_3\text{R}$  signalling. **(A)** Activation of Group 1 mGluRs triggers  $\text{IP}_3\text{R}$ -dependent  $\text{Ca}^{2+}$  release from ER stores. When synaptic activation is restricted to a few synapses,  $\text{Ca}^{2+}$  signalling is restricted to the spine head of active synapses containing ER content. Such  $\text{Ca}^{2+}$  signalling can be associated with LTD. **(B)** Activation of a cluster of synapses, by contrast, can trigger a propagating  $\text{Ca}^{2+}$  wave.  $\text{Ca}^{2+}$  waves are supported by  $\text{IP}_3$  production at a number of active synapses along the dendrite, and are augmented by  $\text{Ca}^{2+}$  influx from other sources, including NMDARs and VGCCs, which act synergistically with  $\text{IP}_3$  to promote  $\text{IP}_3\text{R}$  activity. These waves are thought to mediate the spread of postsynaptic LTD, but may also be important for triggering dendritic protein synthesis to support LTP expression.

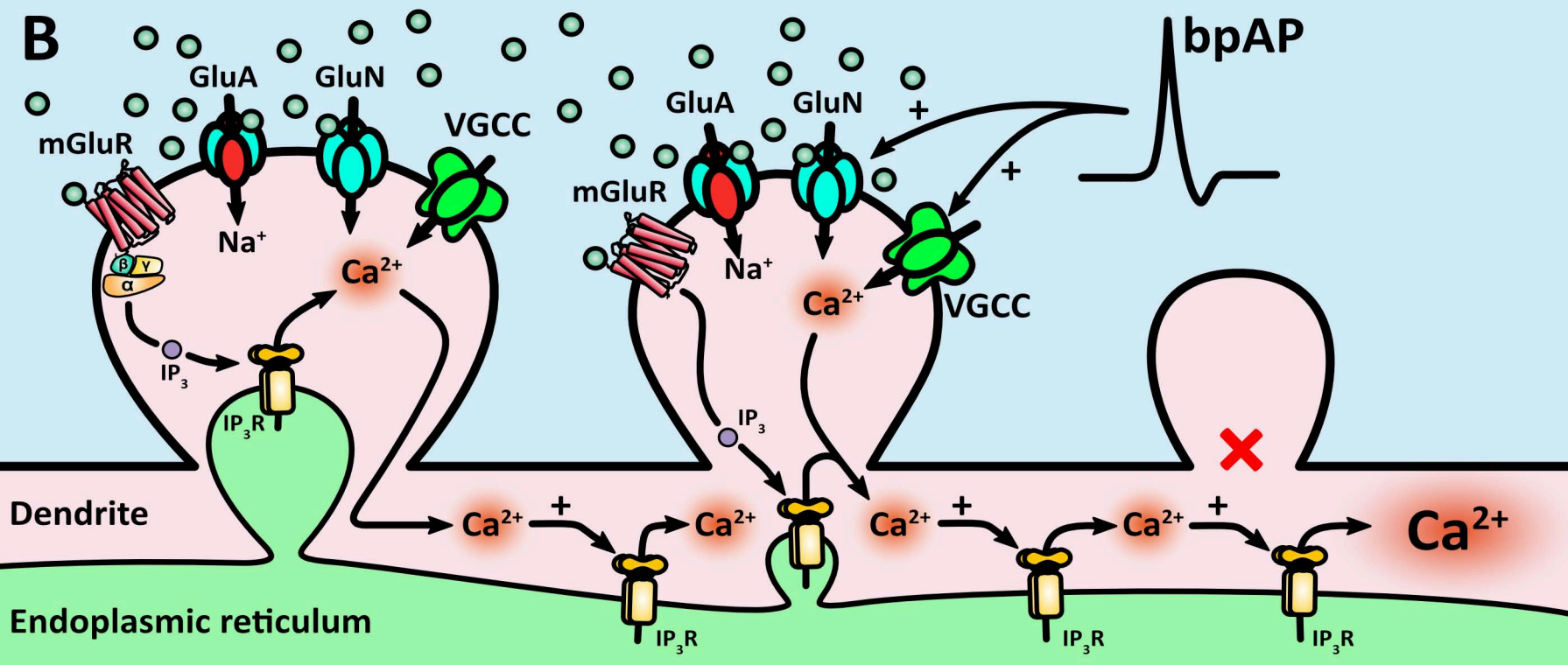
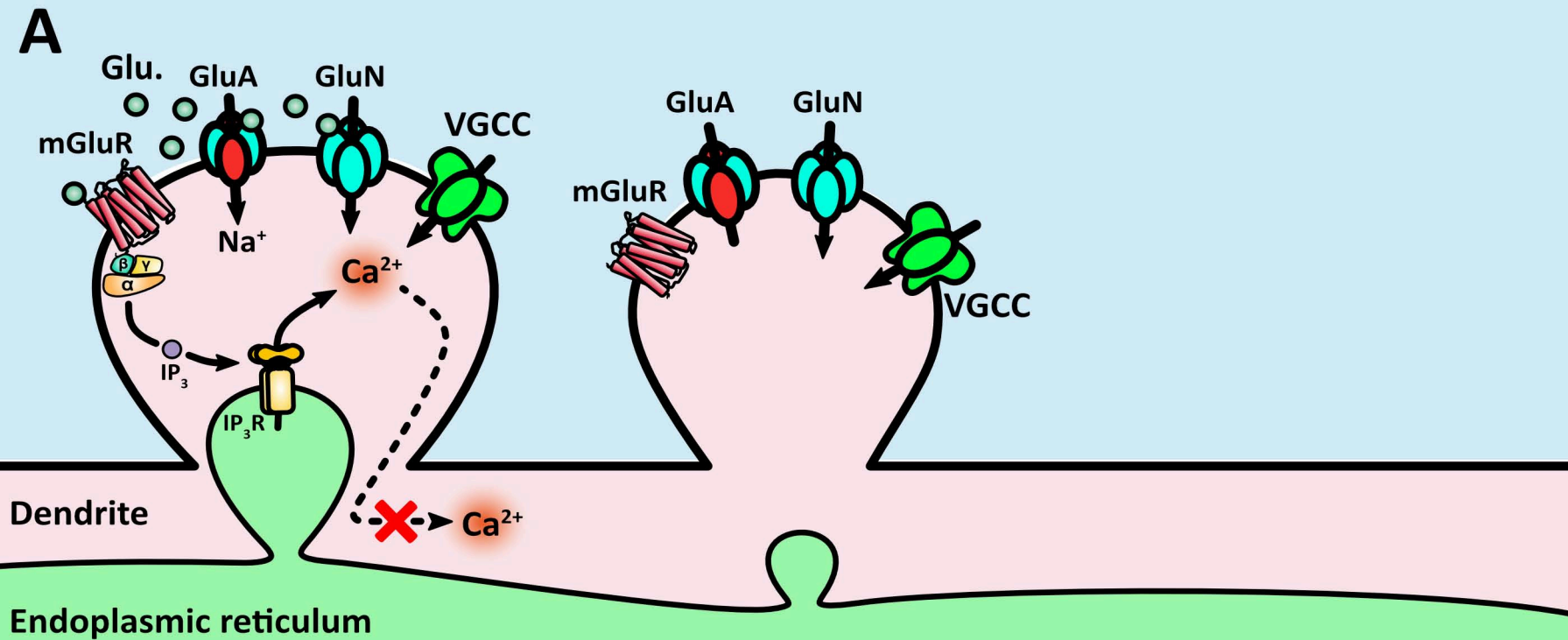
**Figure 3.** Presynaptic RyR signalling. (Left) AP-induced  $\text{Ca}^{2+}$  influx from presynaptic VGCCs triggers further  $\text{Ca}^{2+}$  release from RyR-gated ER stores in boutons. This  $\text{Ca}^{2+}$  release mediates short-term presynaptic facilitation (i.e. transient increases in  $P_r$ ) during short bursts of presynaptic activity. (Right). APs can also trigger  $\text{Ca}^{2+}$  release from presynaptic lysosomes. This release is triggered by  $\text{Ca}^{2+}$  influx from VGCCs, and requires coupling of the lysosome to RyR-gated ER stores; though, the exact nature of VGCC-lysosome-RyR interactions are not known. The function of lysosomal  $\text{Ca}^{2+}$  release is also not well understood.

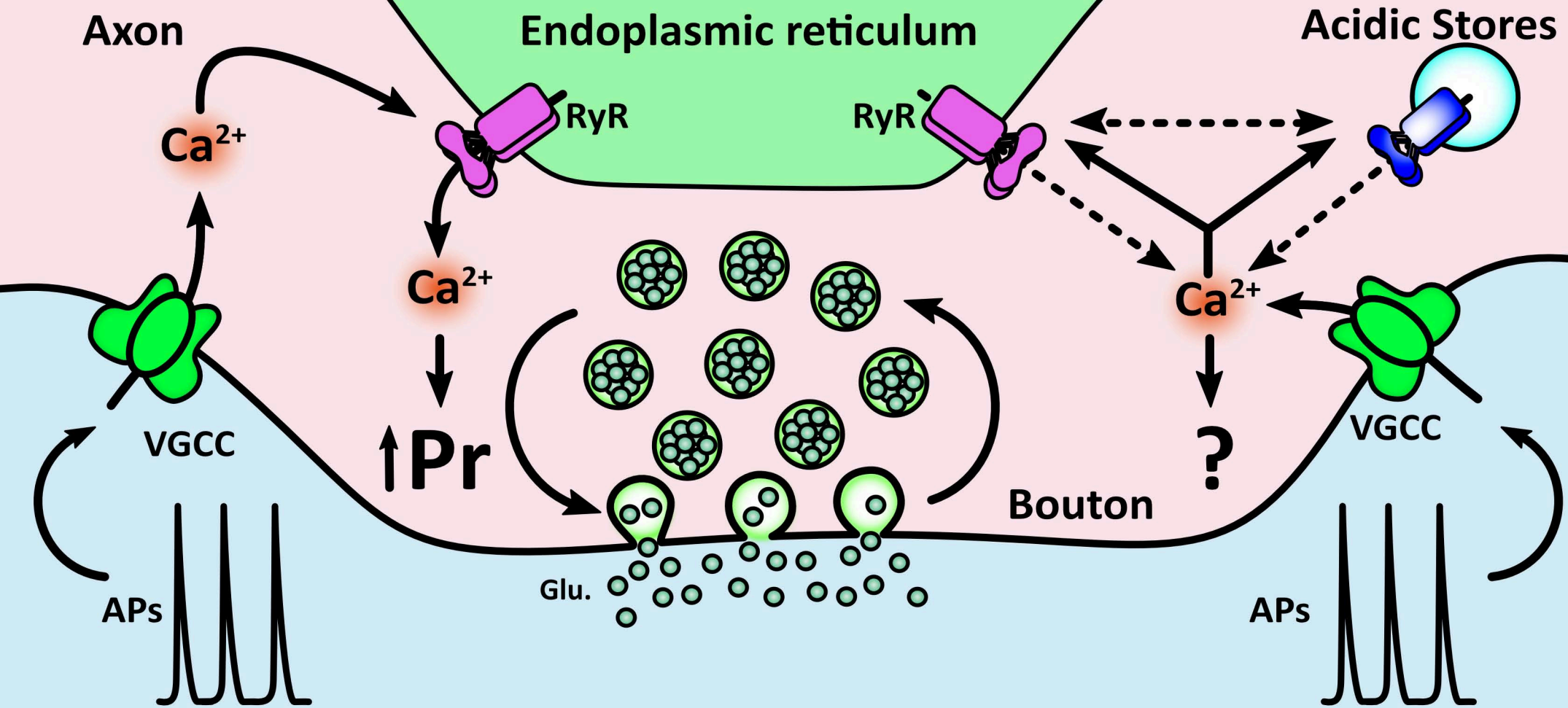
**Figure 4.** Presynaptic mitochondrial signalling. Elevations in cytosolic  $\text{Ca}^{2+}$  during high frequency activity are buffered by mitochondria via the actions of a mitochondrial  $\text{Ca}^{2+}$  uniporter. This buffering limits vesicle depletion during rigorous activity, thereby preventing post-tetanic depression at the Calyx of Held and NMJ. At the NMJ, following prolonged high frequency activity, a relatively long-lasting elevation in cytosolic  $\text{Ca}^{2+}$  is observed, caused by slow release of sequestered  $\text{Ca}^{2+}$  from mitochondrial stores. This  $\text{Ca}^{2+}$  supports the post-

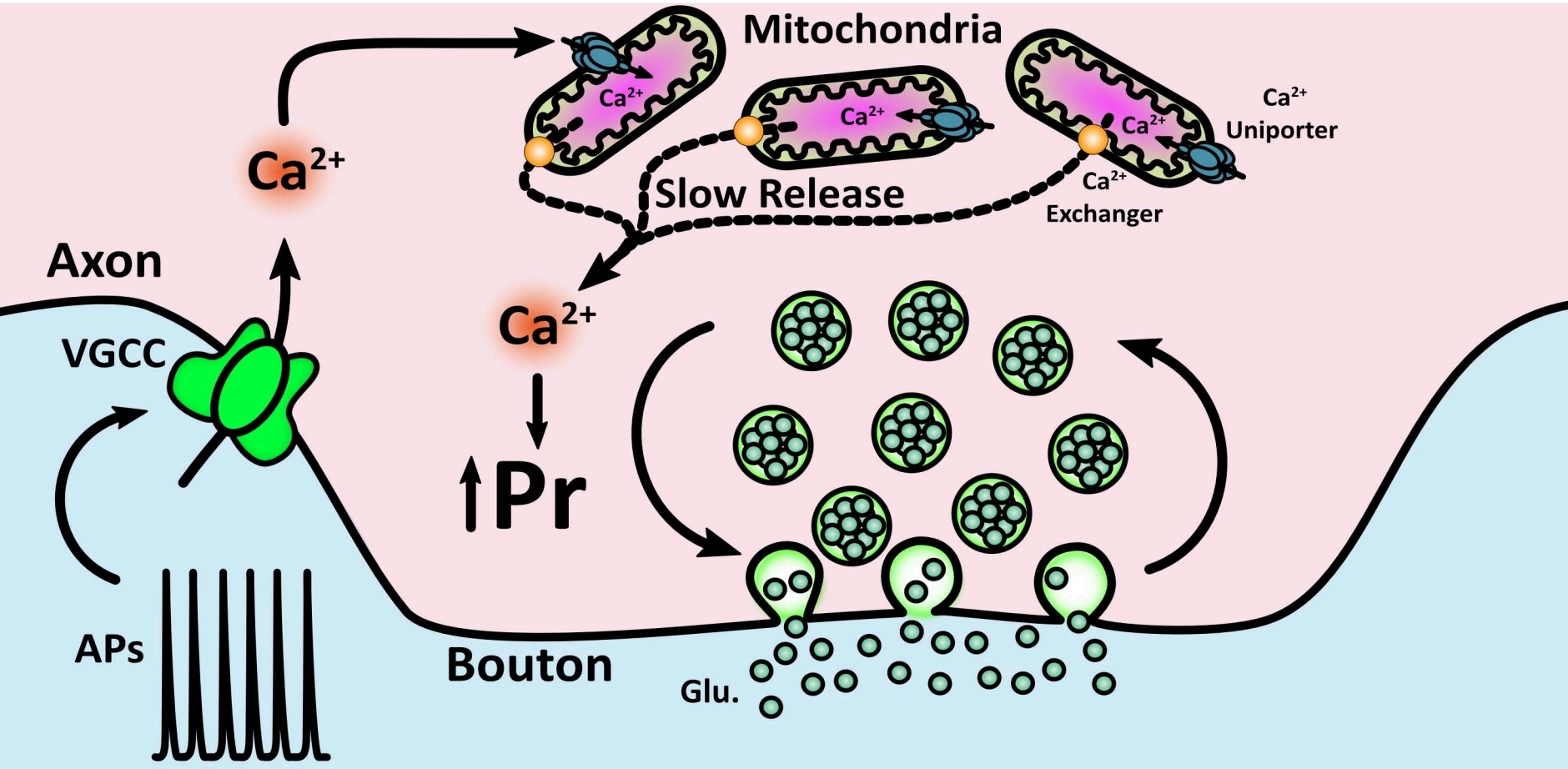
tetanic potentiation of transmitter release. It is not known whether similar functions are supported by mitochondria at hippocampal synapses during high frequency activity.

**Figure 5.** Postsynaptic lysosomal signalling is essential for the maintenance of postsynaptic LTP. 1) bpAPs trigger  $\text{Ca}^{2+}$  influx from dendritic VGCCs. 2) This in turn elicits  $\text{Ca}^{2+}$  release from the lysosomes via a NAADP-dependent mechanism. 3) Lysosomal  $\text{Ca}^{2+}$  release triggers fusion of the lysosome with the plasma membrane, resulting in the release of its intracellular contents into the extracellular matrix (ECM). 4) Lysosomal Cathepsin B, which retains its enzymatic activity in the pH neutral environment of the ECM, activates MMP-9 by cleaving its endogenous inhibitor, TIMP-1. 5) Active MMP-9 triggers ECM remodelling and enables maintenance of postsynaptic LTP.

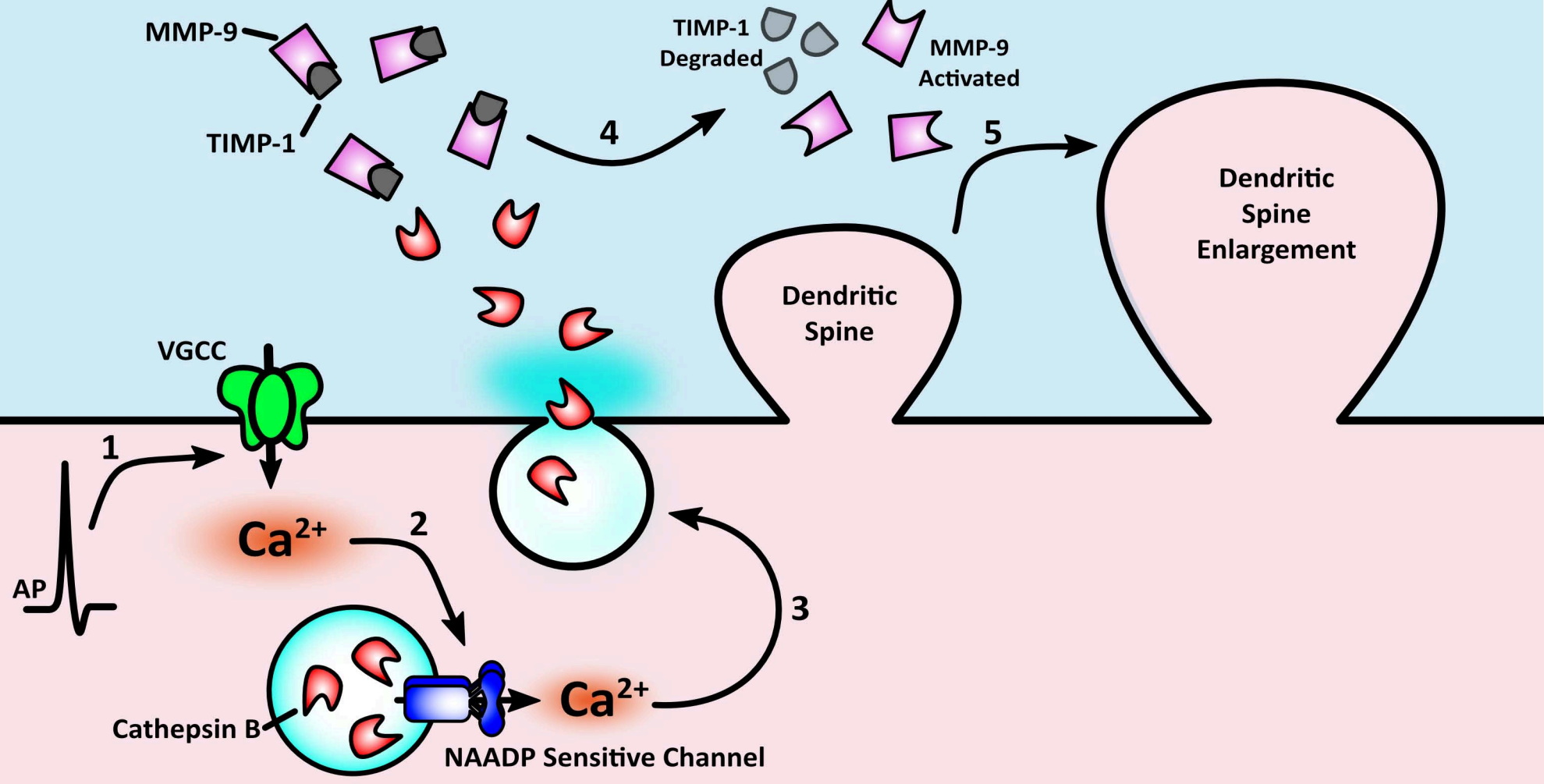












**Table 1. Ca<sup>2+</sup> store signalling and function at hippocampal synapses**

Ca <sup>2+</sup> Store	Ca <sup>2+</sup> Signal	Minimal trigger	Effect on synaptic transmission/plasticity	Stimulation condition(s) under which effect is observed	Key References
RyR <sub>pre</sub>	Localized Ca <sup>2+</sup> spark in boutons	VGCC activation by single APs	Modest, basal increase in P <sub>r</sub>	Single APs	(Cabezas and Buno 2006)
			Required for NMDAR-dependent LTD <sub>pre</sub>	Prolonged, low frequency stimulation (1-5 Hz)	(Reyes and Stanton 1996, Unni, Zakharenko et al. 2004)
			Required for short-term, presynaptic facilitation/paired pulse facilitation	-Moderate frequency stimulation (10-20Hz; minimum of 2 APs) -No effect at postsynaptically silent synapses	(Emptage, Reid et al. 2001, Cabezas and Buno 2006, Zhang, Wu et al. 2009)
RyR <sub>post</sub>	Localized Ca <sup>2+</sup> spark in spines, which can spread to the dendrite in younger tissue	-NMDAR activation by unquantal glutamate release  -VGCC activation by APs delivered at high frequency (100Hz)	Required for NMDAR-dependent LTD <sub>post</sub>	-Low frequency stimulation (1-5 Hz) -Anti-causal post/pre pairing with afferent stimulation (STDP)	(Wang, Wu et al. 1996, Futatsugi, Kato et al. 1999, Nishiyama, Hong et al. 2000)
			Required for NMDAR-dependent LTP <sub>post</sub>	-Moderate stimulation (single, but not multiple, trains of tetanic/theta burst stimulation) -Causal pre/post pairing (STDP)	(Behnisch and Reymann 1995, Balschun, Wolfer et al. 1999, Raymond and Redman 2002, Raymond and Redman 2006, Kwon and Sabatini 2011, Lee, Soares et al. 2016)
			Possibly inhibits L-VGCC-dependent and NMDAR-independent LTP <sub>pre</sub>	-High frequency stimulation (100 Hz)	(Futatsugi, Kato et al. 1999, Dittmer, Wild et al. 2017) (Dittmer, Wild et al. 2017)
IP <sub>3</sub> R <sub>post</sub>		Group 1 mGluR activation by	Required for mGluR-dependent LTD <sub>post</sub>	-Low frequency glutamate uncaging at ER-containing spines (1 Hz)	(Taufiq, Fujii et al. 2005, Holbro, Grunditz et al.



	Localized $\text{Ca}^{2+}$ spark in ER containing spines	uniquantal glutamate release at single synapses		-Low frequency afferent stimulation (1 Hz)	2009, Oh, Hill et al. 2012)
			-Required for depotentiation -Suppresses NMDAR-dependent LTP	Low frequency afferent stimulation (1 Hz)	(Fujii, Matsumoto et al. 2000, Yamazaki, Fujii et al. 2012, Fujii, Yamazaki et al. 2016)
	Dendritic $\text{Ca}^{2+}$ waves/ dendritic spread of $\text{Ca}^{2+}$	Group 1 mGluR activation at a number of synapses.  Signalling is augmented by $\text{Ca}^{2+}$ influx from local NMDARs/VGCCs	Required for heterosynaptic $\text{LTD}_{\text{post}}$ during homosynaptic $\text{LTD}_{\text{post}}$ induction	-Low/moderate stimulation frequencies ( $\leq 5\text{Hz}$ ) -Anti-causal post/pre pairing (STDP)	(Lynch, Dunwiddie et al. 1977, Dunwiddie and Lynch 1978, Nakamura, Nakamura et al. 2000, Nishiyama, Hong et al. 2000, Nagase, Ito et al. 2003)
			Required for heterosynaptic $\text{LTD}_{\text{post}}$ during homosynaptic $\text{LTP}_{\text{post}}$ induction	- High stimulation frequencies (15-100Hz) -Glutamate uncaging at multiple synapses in $\text{Mg}^{2+}$ free conditions	(Lynch, Dunwiddie et al. 1977, Dunwiddie and Lynch 1978, Power and Sah 2014, Oh, Parajuli et al. 2015)
			Required for homosynaptic $\text{LTP}_{\text{post}}$	-Moderate theta burst stimulation (4 trains, but not 1 or 8 trains)	(Raymond and Redman 2002, Raymond and Redman 2006)
			Partially inhibits homosynaptic $\text{LTP}_{\text{post}}$	-Tetanic stimulation (100 Hz) -Causal pre/post pairings (STDP)	(Fujii, Matsumoto et al. 2000, Nishiyama, Hong et al. 2000, Nagase, Ito et al. 2003, Taufiq, Fujii et al. 2005, Yoshioka, Yamazaki et al. 2010)
Mitochondria <sub>pre</sub>	Local elevation of cytosolic $\text{Ca}^{2+}$ in boutons following stimulation	Activation of VGCCs by high frequency ( $\geq 20\text{ Hz}$ ) and prolonged activity ( $\geq 5\text{s}$ )	Required for post-tetanic potentiation	Prolonged ( $\geq 5\text{s}$ ), high frequency ( $\geq 20\text{ Hz}$ ) stimulation ( <i>at Calyx of Held and NMJ</i> )	(Tang and Zucker 1997, David and Barrett 2000)

	Local Ca <sup>2+</sup> sequestration in boutons during stimulation	Observed even with activation of VGCCs by single APs	Prevents post-tetanic depression	High frequency stimulation (100 Hz) ( <i>at Calyx of Held and NMJ</i> )	(David and Barrett 2000, Billups and Forsythe 2002, David and Barrett 2003)
			Unknown/No observable consequence at stimulation frequencies tested	Low frequency stimulation (10Hz) ( <i>at hippocampal synapses and NMJ</i> )	(David and Barrett 2000, Waters and Smith 2003, Ivannikov, Sugimori et al. 2013, Sun, Qiao et al. 2013, Gazit, Vertkin et al. 2016)
Mitochondria <sub>post</sub>	Ca <sup>2+</sup> sequestration in dendrites	NMDAR activation	Not directly examined. Potentially important for NMDAR-dependent LTD <sub>post</sub> induction (see text)	N/A	(Peng, Jou et al. 1998, Kannurpatti, Joshi et al. 2000)
Lysosome <sub>pre</sub>	Boutonal Ca <sup>2+</sup> elevation	VGCC activation by single APs	Unknown	N/A	(McGuinness, Bardo et al. 2007)
Lysosome <sub>post</sub>	Dendritic Ca <sup>2+</sup> elevation	VGCC activation by single bpAPs	Required for the maintenance of NMDAR-dependent LTP <sub>post</sub> by recruiting MMP9 signalling (see text)	Hebbian pairing of synaptic activity with bpAPs	(Padamsey, McGuinness et al. 2017)