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REVIEW ARTICLE

Calcium as a Trigger for Cerebellar Long-Term Synaptic Depression

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Abstract Cerebellar long-term depression (LTD) is a form of long-term synaptic plasticity that is triggered by calcium (Ca^{2+}) signals in the postsynaptic Purkinje cell. This Ca^{2+} comes both from IP3-mediated release from intracellular Ca^{2+} stores, as well as from Ca^{2+} influx through voltage-gated Ca^{2+} channels. The Ca^{2+} signal that triggers LTD occurs locally within dendritic spines and is due to supralinear summation of signals coming from these two Ca^{2+} sources. The properties of this postsynaptic Ca^{2+} signal can explain several features of LTD, such as its associativity, synapse specificity, and dependence on the timing of synaptic activity, and can account for the slow kinetics of LTD expression. Thus, from a Ca^{2+} signaling perspective, LTD is one of the best understood forms of synaptic plasticity.

Keywords Synaptic plasticity · Purkinje cell · IP3 · Ca^{2+} channel · Protein kinase C

Introduction

Long-lasting changes in the strength of synaptic transmission are widely thought to provide the basis for persistent changes in the brain that occur during learning and memory, development, and some pathological conditions. Many forms of long-term synaptic plasticity are triggered by postsynaptic calcium (Ca^{2+}) signals arising from specific patterns of synaptic activity. Ca^{2+} is a rich source of biological information because of the diverse sources of Ca^{2+}, the precise spatial localization of Ca^{2+} signaling machinery, the complex spatial and temporal dynamics of Ca^{2+} signals, interactions among Ca^{2+} signals and other signaling pathways, and the diversity of targets regulated by Ca^{2+}. Thus, elucidating the role of Ca^{2+} in triggering long-term synaptic plasticity requires an understanding of these properties.

Cerebellar long-term depression (LTD) is a form of Ca^{2+}-dependent long-term synaptic plasticity. Although diverse forms of LTD are found throughout the brain—often involving different types of signaling—for convenience, in this article, we will use "LTD" to refer specifically to LTD at synapses between parallel fibers (PFs) and Purkinje cells in the cerebellum. LTD is induced by simultaneous and repetitive activity at the two excitatory inputs onto Purkinje neurons, the PFs and the climbing fiber (CF) (Fig. 1a) [1–6]. LTD is expressed as a long-lasting decrease in the strength of transmission at the PF synapse, due to a reduction in AMPA-type glutamate receptors (AMPARs) on the dendritic spines of the postsynaptic Purkinje cell (Fig. 1b) [7–10]. This decrease in synaptic strength develops gradually, over minutes, and lasts for hours or much longer (Fig. 1c). LTD can also be induced at CF synapses [11]. While many studies support the idea that LTD plays an important role in certain forms of motor learning [12–17], the precise nature of this role remains an area of intense investigation and controversy [18, 19]. In contrast, at the cellular level, there is an emerging understanding of the mechanisms through which Ca^{2+} signaling brings about this long-lasting change in synaptic function (Fig. 1b). The goal of our article is to review our

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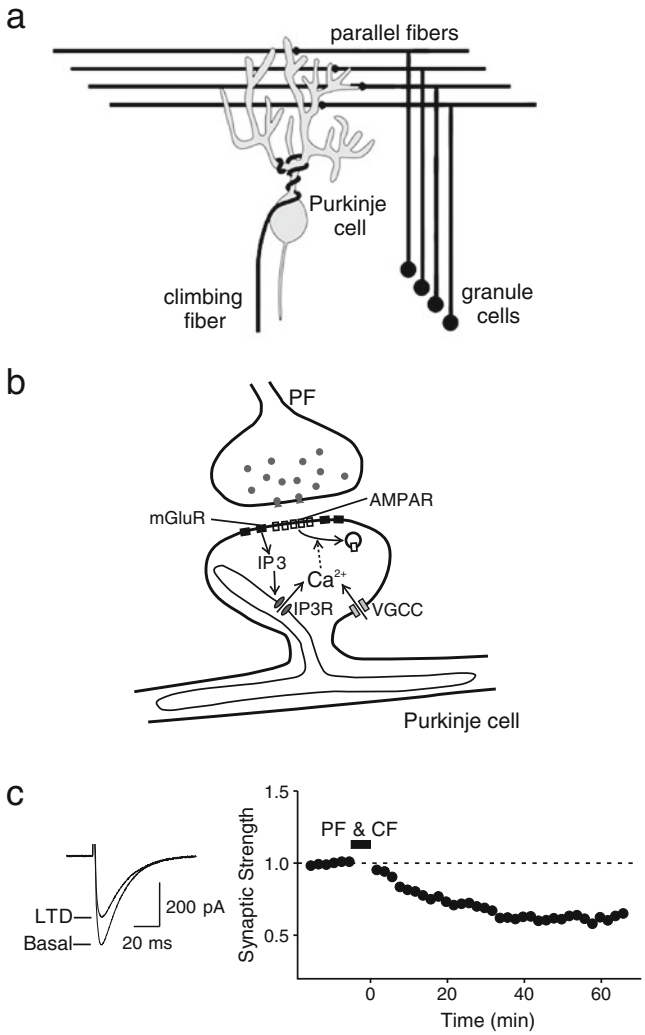

Fig. 1 Cerebellar LTD is induced by conjunctive stimulation of the CF and PFs. **a** Schematic illustration of the synaptic connections between the CF and PFs and a Purkinje cell. Each Purkinje cell is innervated by a single CF on the soma and proximal dendrites, while it is innervated by many PFs on spines of the distal dendrites. **b** LTD occurs at the synapse between a PF and a Purkinje cell. LTD is induced by an increase in spine $[Ca^{2+}]_i$ produced by Ca^{2+} entry through VGCCs and subsequent Ca^{2+} release from intracellular stores by IP3Rs. LTD is expressed as a reduction in AMPARs on dendritic spines of the postsynaptic Purkinje cell. **c** *Left*, Superimposed traces of PF-EPSC before and following LTD induction. *Right* Time course of normalized PF-EPSC amplitude before and after LTD is induced. Note that LTD is gradually expressed after conjunctive PF/CF stimulation

current understanding of the postsynaptic Ca^{2+} signaling processes underlying the triggering of LTD at PF synapses and to highlight some of the open questions that remain.

Ca^{2+} Is Required for LTD Induction

LTD has long been known to depend on elevation of Ca^{2+} concentration ($[Ca^{2+}]_i$) within the postsynaptic Purkinje cell. The earliest evidence for this came from studies showing that synaptic activity that induces LTD also increases $[Ca^{2+}]_i$ in Purkinje cells [5, 20, 21]. Postsynaptic introduction of the Ca^{2+} chelators EGTA [22, 23] or BAPTA [5] blocks LTD, indicating that a rise in postsynaptic $[Ca^{2+}]_i$ is necessary for LTD induction. Increases in postsynaptic $[Ca^{2+}]_i$ are also sufficient to induce LTD: elevating $[Ca^{2+}]_i$ to suprathreshold levels, by activation of IP3-mediated Ca^{2+} release via photolysis of caged IP3 [24] or by photolysis of caged Ca^{2+} [25, 26], can induce LTD (see Fig. 3a below). Several stimuli that produce a subthreshold rise in postsynaptic $[Ca^{2+}]_i$—such as activation of Ca^{2+} channels via depolarization [5, 27, 28] or photolysis of caged Ca^{2+} [26, 29, 30]—can be paired with PF activity to induce LTD. Moreover, patterns of PF activity that produce particularly large increases in postsynaptic $[Ca^{2+}]_i$ are sufficient to induce LTD [20, 31]. Together, these findings provide strong evidence that an increase in postsynaptic $[Ca^{2+}]_i$ underlies induction of LTD.

Sources of Ca^{2+} Signals Involved in LTD Induction

Postsynaptic Ca^{2+} signals generated by PF and CF activity play a critical role in LTD induction. PF and CF synaptic inputs onto Purkinje neurons are organized in fundamentally different ways and produce postsynaptic Ca^{2+} signals that are spatially and temporally distinct (Fig. 2). These postsynaptic Ca^{2+} signals are derived from a number of sources, including entry through voltage-gated Ca^{2+} channels (VGCCs), entry through transmitter-gated channels, and release from intracellular stores. The organization of the PF and CF synaptic inputs, localization and regulation of the Ca^{2+} signaling and homeostasis machinery, and interactions among Ca^{2+} signaling pathways together determine how these synaptic inputs interact to generate the Ca^{2+} signals that trigger LTD.

Climbing Fibers Each mature Purkinje neuron receives synaptic input from a single CF arising from the inferior olive, which makes several hundred synaptic contacts on the soma and spines on the proximal dendrites. Activation of the CF evokes large, all-or-none synaptic responses that are mediated primarily by AMPARs [32–34]. The Ca^{2+} permeability of AMPARs in Purkinje cells is relatively low due to the presence of the GluA2 subunit in these receptors. Thus, Ca^{2+} influx directly through AMPARs contributes little to postsynaptic Ca^{2+} signals. However, the massive AMPAR-mediated depolarization evokes a regenerative action potential that propagates into the dendrites, known as the complex spike [35]. The complex spike is primarily generated by activation of both P/Q type VGCCs and Na^+ channels [36], although it also includes contributions from

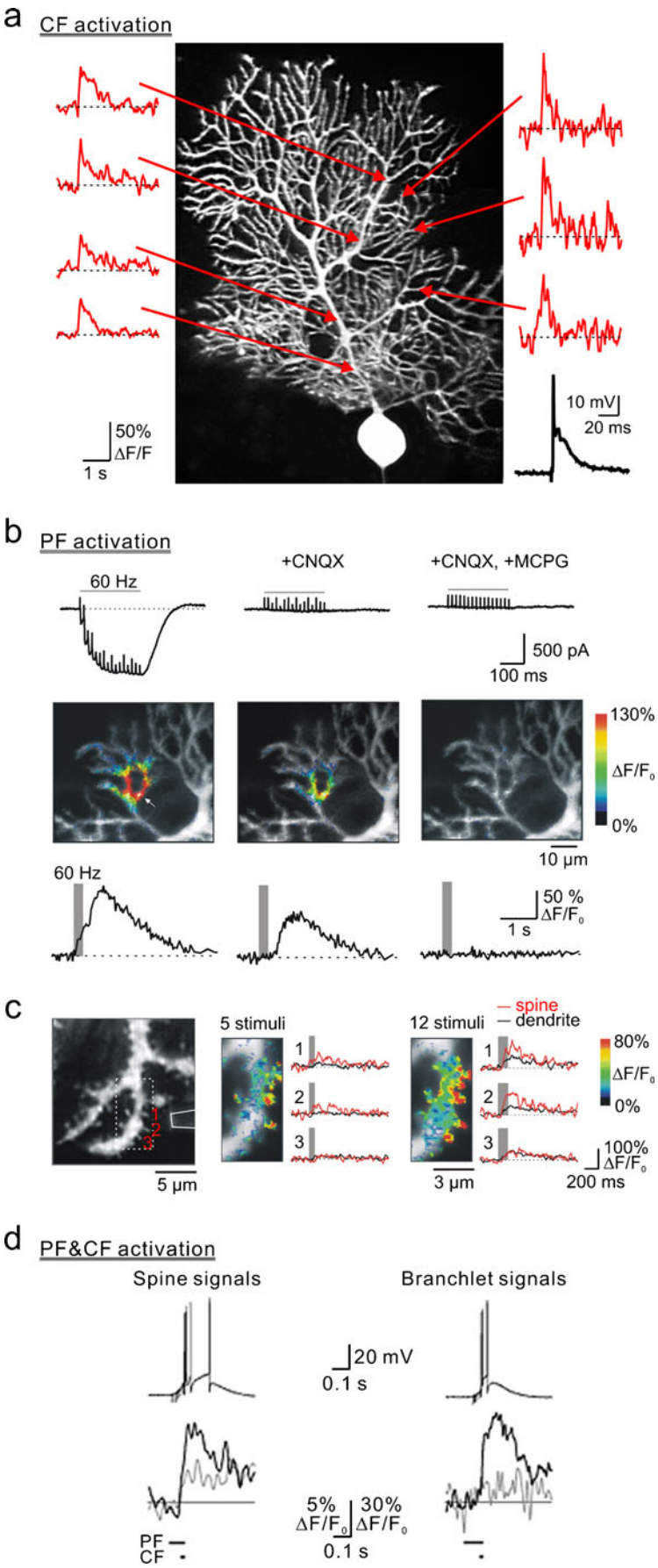

Fig. 2 $[Ca^{2+}]_i$ increases produced in Purkinje cells by CF, PF, and conjunctive PF/CF activity. **a** Purkinje cell responses to CF activation. $[Ca^{2+}]_i$ increases throughout the entire dendritic arbor of the Purkinje cell. **b** Purkinje cell responses to a burst of PF activity. The $[Ca^{2+}]_i$ increase consists of two components. An initial fast component is mediated by AMPARs and is blocked by CNQX, an AMPAR antagonist. A subsequent slow component is mediated by mGluRs and is blocked by MCPG, an mGluR antagonist [24]. **c** With only a few PF stimuli, the postsynaptic $[Ca^{2+}]_i$ increase is restricted to postsynaptic spines. With an increased number of PF stimuli, the $[Ca^{2+}]_i$ increase is larger in postsynaptic spines and also extends to adjoining dendritic shafts [24]. **d** Purkinje cell responses to conjunctive stimulation of the CF and PFs. The $[Ca^{2+}]_i$ increase produced by paired PF/CF activity (*black traces*) is larger than the sum of the $[Ca^{2+}]_i$ increases by CF and PF activity alone (*gray traces*) [21]

low-threshold VGCCs, mGluR-TRPC channels, and Kv3.3 potassium channels [37–40]. As a result of VGCC activation, CF activity produces a widespread increase in dendritic $[Ca^{2+}]_i$ (Fig. 2a) [5, 41, 42]. CF activity can also activate mGluRs [43–45] and NMDARs [46, 47], with NMDARs reportedly contributing to CF-mediated $[Ca^{2+}]_i$ increases [48]. Because dendritic Ca^{2+} spikes generated by somatic depolarization can supplant the requirement for CF activity [5], it is widely accepted that the signal produced by CF activity that is essential for the induction of LTD is a depolarization-mediated increase in $[Ca^{2+}]_i$. However, regardless of their sources, CF-mediated Ca^{2+} signals are insufficient to trigger LTD. Given that a rise in postsynaptic $[Ca^{2+}]$ is sufficient to cause LTD [26], we can conclude that additional Ca^{2+} signals provided by PF activity are also required.

Parallel Fibers Each mature Purkinje cell receives synaptic contacts from over 100,000 PFs, the axons of granule cells, at synapses located on dendritic spines of higher order dendritic branches. At PF synapses, released glutamate activates postsynaptic AMPARs and mGluRs on spines (Fig. 2b). Postsynaptic NMDARs, however, do not contribute to PF-Purkinje cell synaptic transmission [34, 49]. PF activity generates Ca^{2+} signals that have complex spatial and temporal properties, the specific features of which depend on the pattern of PF activity (Fig. 2b, c). Unlike the widespread all-or-none Ca^{2+} responses to CF activity, PF Ca^{2+} signals can be restricted to individual postsynaptic spines (Fig. 2c) [21, 24] or, with activation of a larger number of PFs, can extend beyond individual spines to encompass larger dendritic domains that can extend over approximately 10 μm (Fig. 2b) [20, 21, 24, 50–52].

Responses to single bouts of PF activity are mediated by AMPARs: Activation of PFs with a single stimulus produces a graded increase in EPSP amplitude with increasing stimulus intensity [32]. Recruitment of a sufficient number of PF inputs depolarizes the postsynaptic membrane potential to a level that activates VGCCs, causing a rapid increase in $[Ca^{2+}]_i$ [50]. These postsynaptic Ca^{2+} signals are spatially restricted to small spino-dendritic compartments. Repetitive activation of PFs also activates mGluR1α, which has unambiguously been shown to be essential for LTD [53–55]. mGluR1 activation generates slower and more complex postsynaptic signals than those associated with AMPAR activation [24, 52, 56]. mGluR1 activates PLCβ4 [57], which produces two intracellular messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). While DAG activates protein kinase C (PKC), an important Ca^{2+} target during LTD (see below), IP3 binds to IP3 receptors (IP3Rs) to stimulate Ca^{2+} release from intracellular stores. The properties of Purkinje cell IP3 receptors are reviewed by Mikoshiba et al. [58] in this issue. Activation of mGluR1 also generates a slow depolarizing membrane conductance that arises a few hundred milliseconds following synaptic activity and decays within a few seconds [56, 59]. The resulting slow EPSP requires TRPC3 channels, which make a relatively small contribution to mGluR1-mediated increases in postsynaptic $[Ca^{2+}]_i$ despite the Ca^{2+} permeability of these channels [60]. A role for TRPC3 in LTD has not yet been established.

Several lines of evidence demonstrate that IP3-induced Ca^{2+} release at PF synapses is both necessary and sufficient to generate the postsynaptic Ca^{2+} signals required to trigger LTD. IP3R-mediated Ca^{2+} release is necessary because blockade of IP3Rs with the IP3R antagonist heparin [61] or an anti-IP3R antibody [62] prevents the induction of LTD. Furthermore, LTD is absent in Purkinje cells from type I IP3R null mice [62]. Photolysis of caged IP3 can induce LTD [24], showing that IP3-evoked Ca^{2+} release is sufficient for LTD. Moreover, IP3-mediated Ca^{2+} release must occur within postsynaptic spines because LTD is abolished in mice and rats where smooth ER and IP3 receptors are selectively lost from spines [25]. This defect in LTD is due to the loss of Ca^{2+} release within spines because it is rescued by photolysis of caged Ca^{2+} at PF synapses. Similarly, in mGluR1 null Purkinje cells, pairing IP3 uncaging with depolarization is sufficient to rescue LTD [63]. Together, these findings have clearly established that IP3-mediated Ca^{2+} release within postsynaptic dendritic spines following activation of mGluRs by PF activity plays a central role in generating the Ca^{2+} signals that trigger LTD. Furthermore, even though IP3 can spread and act at a distance [24], Ca^{2+} must be released locally within dendritic spines to produce LTD.

In summary, the evidence to date suggests that the critical postsynaptic Ca^{2+} signaling events underlying LTD are the spatial and temporal overlap of CF-induced Ca^{2+} entry into dendrites through VGCCs and the PF-induced activation of mGluRs and subsequent IP3-mediated Ca^{2+} release within PF dendritic spines.

The Postsynaptic Ca^{2+} Signal That Produces LTD

To understand the signaling mechanisms underlying LTD, it is fundamentally important to define the properties of the postsynaptic $[Ca^{2+}]_i$ increase that triggers LTD. LTD is induced by simultaneous low frequency (e.g., 1 Hz) activity of the CF and PF synapses for several minutes, while similar amounts of activity at CF or PF synapses alone or a single bout of PF/CF pairing do not yield LTD. Because of limitations caused by the dynamic range of Ca^{2+} indicators and the temporal and spatial resolution of fluorescence

microscopes, it is difficult to precisely define the amplitude, time course, and spatial range of the postsynaptic Ca^{2+} signals produced by PF, CF, and paired PF/CF activity. Nonetheless, several studies have estimated the key properties of these Ca^{2+} signals.

Ca^{2+} signals evoked by CF activity are transient, rising within milliseconds and decaying in <1 s (Fig. 2a). These signals are moderate in amplitude, reaching several hundred nanomolar in dendritic shafts and somewhat higher levels in dendritic spines [5, 64, 65]. The Ca^{2+} signals produced by PF activity vary widely depending on the number and frequency of PF stimuli, ranging from several hundred nanomolar with a single PF stimulus up to nearly 10 μm with bursts of PF activity (Fig. 2b, c). While Ca^{2+} signals produced by single PF stimuli decay within 1 s, Ca^{2+} signals produced by bursts of PF activity last several seconds (Fig. 2b) [20, 21, 24, 50–52, 66].

Pairing PF and CF activity produces supralinear increases in postsynaptic $[Ca^{2+}]_i$ at activated PF synapses that are larger in amplitude than the linear sum of the Ca^{2+} signals produced by PF and CF activity alone (Fig. 2d). These Ca^{2+} signals range from a few micromolar to more than 10 μM, depending on the pattern of activity [21, 66]. With repetitive pairing of PF and CF activity, repetitive Ca^{2+} transients are superimposed on an elevated $[Ca^{2+}]_i$ plateau and decay back to baseline levels within a minute after stimulation is terminated [21, 24, 26, 31].

In summary, the complex spatiotemporal dynamics of postsynaptic Ca^{2+} signals produced during synaptic activity makes it difficult to define the precise concentration and duration of the $[Ca^{2+}]_i$ increase required for LTD. Nonetheless, it is safe to conclude that postsynaptic $[Ca^{2+}]_i$ rises to the micromolar range for at least a few seconds when LTD is induced by paired PF and CF activity.

To define the precise postsynaptic $[Ca^{2+}]_i$ requirements for LTD, local photolysis of caged Ca^{2+} has been used to control the concentration and duration of the $[Ca^{2+}]_i$ increase in Purkinje cell dendrites [26]. With this approach, elevating $[Ca^{2+}]_i$ has been found to induce synaptic depression without requiring any of the other signals produced by PF or CF synaptic activity (Fig. 3a). This Ca^{2+}-induced depression of PF synapses shares many properties with LTD induced by synaptic activity and can occlude LTD induced by synaptic activity. These similarities indicate that an increase in $[Ca^{2+}]_i$ is sufficient to produce LTD in the absence of PF or CF activity.

Quantification of the relationship between peak $[Ca^{2+}]_i$ and the amount of LTD reveals that the $[Ca^{2+}]_i$ required to trigger LTD is in the range of several micromolar (Fig. 3b) [26]. This relationship is sigmoidal and highly cooperative, which results in a sharp threshold in the $[Ca^{2+}]_i$ required to induce LTD. Moreover, the Ca^{2+} sensitivity of LTD is a dynamic process that depends on how long $[Ca^{2+}]_i$ is

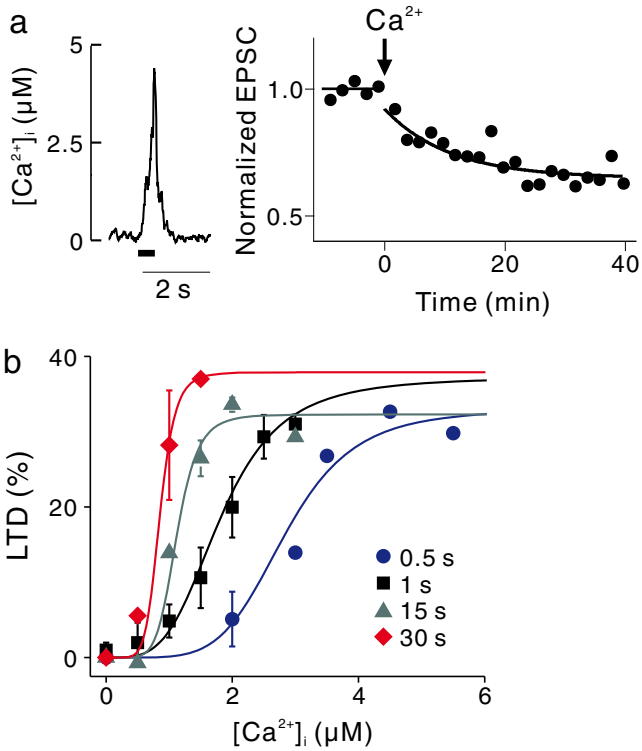

Fig. 3 Postsynaptic $[Ca^{2+}]_i$ increase produced by local photolysis of caged Ca^{2+} is capable of triggering LTD. **a** *Left* Transient $[Ca^{2+}]_i$ increase induced by photolysis of caged Ca^{2+} (0.5-s duration). *Right* Time course of LTD induced by the transient $[Ca^{2+}]_i$ increase (*right*). **b** The relationship between peak $[Ca^{2+}]_i$ and LTD for $[Ca^{2+}]_i$ increases in different durations [26]

elevated in the postsynaptic Purkinje cell: higher $[Ca^{2+}]_i$ is required for brief $[Ca^{2+}]_i$ increases, while lower $[Ca^{2+}]_i$ is sufficient for longer $[Ca^{2+}]_i$ increases (Fig. 3b). For example, the peak $[Ca^{2+}]_i$ required for half-maximal LTD is ~1.8 μM with an increase in $[Ca^{2+}]_i$ that lasts for 1 s, while it is ~0.9 μM for a 30-s long $[Ca^{2+}]_i$ increase. This dependence on both the amplitude and the duration of postsynaptic Ca^{2+} signals undoubtedly underlies the selective induction of LTD by specific patterns of synaptic activity (see below). Furthermore, this dependence on the duration of the $[Ca^{2+}]_i$ increase indicates that the LTD induction mechanism can integrate Ca^{2+} signals over time. However, the total integrated amount of Ca^{2+} required for short $[Ca^{2+}]_i$ increases is less than that required for long $[Ca^{2+}]_i$ increases [26], indicating that this integration mechanism becomes less effective—or "leaky"—during prolonged increases in $[Ca^{2+}]_i$. These properties have important implications for understanding the steps that lie downstream from Ca^{2+} in the molecular cascade that triggers LTD.

It is interesting to compare the postsynaptic Ca^{2+} requirements of cerebellar LTD to that of well-studied forms of long-lasting synaptic plasticity in the hippocampus. In hippocampal CA1 pyramidal neurons, brief eleva-

tion of $[Ca^{2+}]_i$ to approximately 10 μM induces LTP, while elevating $[Ca^{2+}]_i$ to 0.8 μM for 60 s produces LTD [67]. This influence of the magnitude of postsynaptic $[Ca^{2+}]_i$ elevation on the direction of synaptic plasticity is consistent with the BCM rule [68] It has been proposed that this relationship is reversed in cerebellar Purkinje cells [69], presumably due to differences in the signal transduction mechanisms engaged during LTD and LTP in the two cell types [69]. However, the direction of synaptic plasticity is not always opposite in the two cells. Quantitative analysis reveals that while cerebellar LTD indeed is induced by relatively large and brief rises in $[Ca^{2+}]_i$ (e.g., 5 μM for 0.5 s) in Purkinje cells, increasing $[Ca^{2+}]_i$ to 0.9 μM for 30 s also causes LTD (Fig. 3b). Thus, it is likely that both cerebellar and hippocampal LTD can be triggered by the same "low" postsynaptic Ca^{2+} signal (0.8 μM for 60 s).

Ca^{2+} Signaling Underlying LTD Properties

Here, we discuss how the characteristic properties of LTD may emerge from the properties of the postsynaptic Ca^{2+} signals described above.

Associativity The requirement for joint PF and CF activity indicates that LTD is an associative form of synaptic plasticity and therefore requires activation of one or more molecular coincidence detectors that are engaged during paired PF/CF activity, but not during the activity of either synapse alone. The supralinear increases in postsynaptic $[Ca^{2+}]_i$ produced by pairing PF and CF activity indicates that these coincidence detectors act, at least in part, via Ca^{2+} signaling. Two coincidence detection mechanisms are known to generate supralinear $[Ca^{2+}]_i$ increases during conjoint activation of PF and CF synapses. Both mechanisms can generate peak $[Ca^{2+}]_i$ increases that are >10 μM [21], which is well within the range that is known to trigger LTD based on the Ca^{2+} uncaging experiments.

IP3Rs are widely thought to serve as a coincidence detector for LTD induction [70]. IP3 and Ca^{2+} act as co-agonists for the activation of Ca^{2+} release from IP3Rs, so that IP3-mediated Ca^{2+} release is potentiated by an increase in $[Ca^{2+}]_i$ [71–73]. As a result, the convergent production of IP3 at active PF synapses and generation of Ca^{2+} influx by CF activity could allow IP3Rs to act as molecular coincidence detectors in Purkinje cells. Experimental evidence supports the idea that such a mechanism causes supralinear amplification of IP3R-mediated Ca^{2+} release at PF synapses and, thus, yields an elevation of $[Ca^{2+}]_i$ that is sufficient to induce LTD [21, 65].

A second potential form of coincidence detection is based on Ca^{2+} entry through VGCCs. Due to the nonlinear voltage-dependence of the P/Q type VGCCs found in Purkinje cells, the widespread depolarization produced by CF-mediated complex spikes interacts with the local depolarization produced by PF activity to generate supralinear activation of VGCCs. This, in turn, enhances the magnitude of $[Ca^{2+}]_i$ signals at PF synapses during paired PF/CF activity [21, 66].

The relative contribution of these two coincidence detection mechanisms to Ca^{2+} signaling during LTD induction depends on the pattern of PF synaptic activity. When weak PF activity is paired with CF activity, supralinearity appears to rely primarily on mGluR-mediated Ca^{2+} release by IP3Rs. In contrast, when strong PF activity is paired with CF activity, supralinearity appears to rely on both mGluR-mediated Ca^{2+} release and depolarization-mediated Ca^{2+} entry (Fig. 4a) [21, 66]. This distinction seems to arise because weak PF activity produces only small depolarizations of the membrane potential of Purkinje cell dendritic branches, while strong PF activity produces larger and more widespread depolarization.

Synapse Specificity LTD was long thought to be input specific, or homosynaptic, with synaptic depression occurring only at the activated PF synapses [3, 6, 74]. While this appears to be true for LTD induced by pairing weak PF activity with CF activity [21, 75], subsequent investigation has shown that pairing stronger patterns of PF synaptic activity with CF activity causes LTD to spread to nearby inactive PF synapses on the same postsynaptic Purkinje cell and thus become heterosynaptic [8, 76].

Because the pattern of synaptic activity also determines the spatial range over which postsynaptic $[Ca^{2+}]_i$ is elevated (see above), it is likely that Ca^{2+} signaling is involved in defining whether LTD is homosynaptic or heterosynaptic. The spread of LTD is not, however, likely to be due to the diffusion of either Ca^{2+} or molecules activated downstream of Ca^{2+}: When photolysis of caged Ca^{2+} is used to directly increase postsynaptic $[Ca^{2+}]_i$ locally, the $[Ca^{2+}]_i$ increase spreads only a few microns beyond the site of photolysis and LTD is restricted to synapses where $[Ca^{2+}]_i$ is elevated (Fig. 4b) [26, 77].

The two mechanisms of Ca^{2+} signaling coincidence detection discussed above are likely to govern, at least in part, the degree of synapse specificity of LTD. Homosynaptic LTD appears to require IP3-dependent coincidence detection that is limited to individual PF spines (Figs. 2c and 4a) [21, 24]. Heterosynaptic LTD may spread via both IP3-dependent Ca^{2+} release and depolarization-mediated Ca^{2+} entry. With strong PF activity, propagation of depolarization [21] and diffusion of IP3 beyond activated PF synapses (Figs. 2b and 4a) [24] are likely to interact with the CF-mediated complex spike to generate both supralinear Ca^{2+} entry through VGCCs and supralinear Ca^{2+} release well beyond activated PF inputs. Indeed, it has been

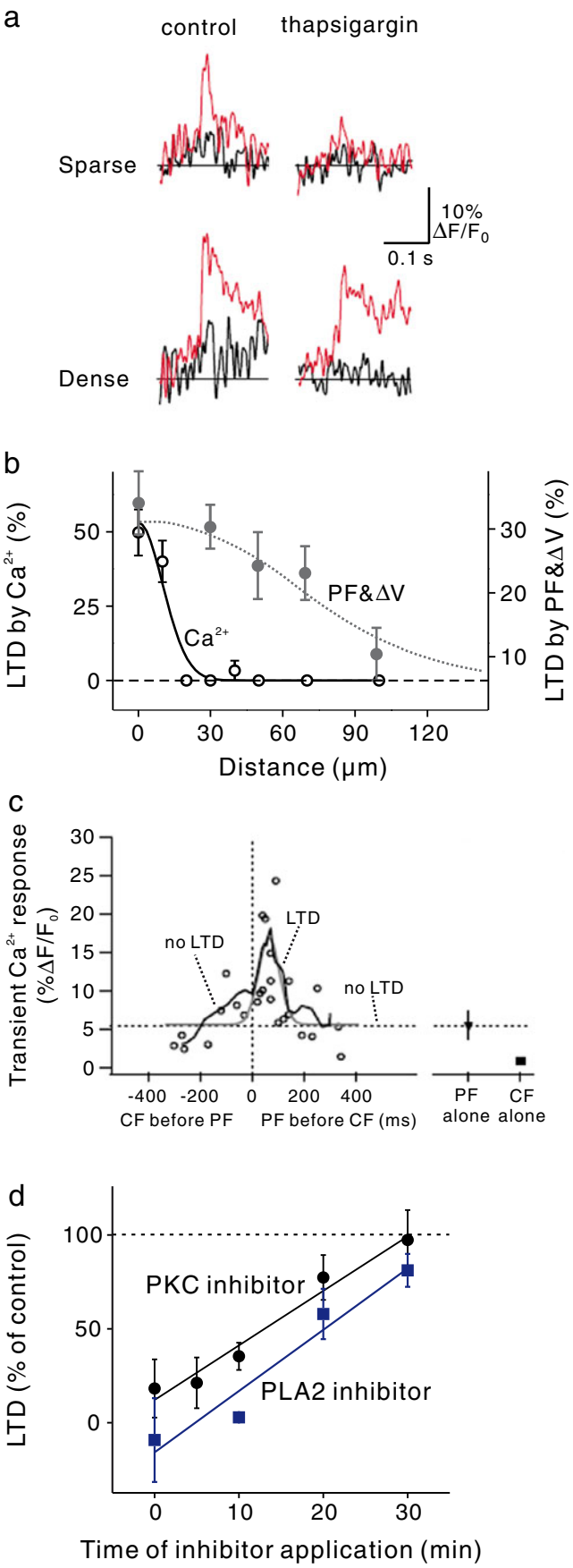

Fig. 4 Ca^{2+} signaling mechanisms underlying several LTD properties **a** The supralinear $[Ca^{2+}]_i$ increase produced by pairing sparse PF activity with CF activity entirely depends on Ca^{2+} release because it is blocked by depleting intracellular Ca^{2+} stores by thapsigargin. In contrast, the $[Ca^{2+}]_i$ increase produced by pairing dense PF activity with CF activity depends on both Ca^{2+} release and Ca^{2+} entry because it is only partially reduced by thapsigargin. *Black and red traces* show responses to PF activity alone and to conjunctive PF/CF activity, respectively [21]. **b** Relationship between the amount of synaptic depression and distance from the site where LTD is induced [8, 26]. Closed circles show heterosynaptic LTD (PF&ΔV), and *open circles* show LTD induced by Ca^{2+} uncaging (Ca^{2+}). **c** Relationship between Ca^{2+} responses, LTD and the timing of PF and CF activity [21]. **d** Relationship between the time of PKC or PLA2 inhibitor application and the degree of LTD impairment [99]

reported that heterosynaptic LTD spreads approximately 50 μm beyond active PF synapses (Fig. 4b) [8, 31] and that the postsynaptic $[Ca^{2+}]_i$ increase spreads at least 10 μm following a single bout of PF/CF pairing [21]. Moreover, it is likely that the spatial extent of postsynaptic Ca^{2+} signaling will be increased further during the repeated PF/CF activity that induces LTD.

NO has also been proposed to mediate the spread of LTD. NO is released during PF activity [78–80] and has long been known to participate in LTD [78, 81–85]. NO is a diffusible signal and has been implicated in the spatial spread of LTD [75, 85]. While the mechanisms involved in NO-mediated spread of LTD are unclear, NO has been shown to cooperate with Ca^{2+} to induce LTD [30] and NO donors both lower the $[Ca^{2+}]_i$ required to induce LTD and enhance IP3-dependent Ca^{2+} release in Purkinje cells [86]. These mechanisms potentially could increase the spatial spread of postsynaptic Ca signaling and/or the spatial spread of LTD induced by a given $[Ca^{2+}]_i$ increase.

In summary, although the mechanisms that govern the spatial extent of LTD are not yet entirely clear, the spatial range of postsynaptic Ca^{2+} signaling and diffusion of NO appear to be involved.

Timing Dependence LTD induction also depends upon the relative timing of PF and CF activity. The precise time window depends on the specific pattern of synaptic activity used to induce LTD. For LTD induced by strong PF activity, LTD is optimally induced when a burst of PF activity precedes CF activity by 50–150 ms [21, 87]. When single PF responses are paired with single CF responses, this time window is 250 ms with 100 paired responses and becomes smaller with larger numbers of paired responses. With 600 pairings, a range of stimulus intervals is effective, even CF activity preceding PF activity [6]. Thus, while the time window for LTD induction is somewhat variable, in general, LTD occurs best when PF activity precedes CF activity by a few hundred milliseconds or less. This timing rule breaks down as the amount of synaptic activity

increases, perhaps because lower $[Ca^{2+}]_i$ is required to induce LTD with a more prolonged $[Ca^{2+}]_i$ increase (see Fig. 3b).

The degree of supralinearity in Ca^{2+} signaling also depends on the relative timing of PF and CF activity. Moreover, both LTD and Ca^{2+} signaling are influenced by the relative timing of PF and CF activity in parallel (Fig. 4c), supporting the idea that the timing dependence of postsynaptic Ca^{2+} signaling plays an important role in determining the timing dependence of LTD. The degree of supralinearity is maximal when PF activity precedes CF activity by 50–250 ms; this is true both for Ca^{2+} signals produced by pairing bursts of weak PF activity with CF activity, which rely on the mGluR-mediated Ca^{2+} signaling pathway, as well as for Ca^{2+} signals produced by pairing bursts of stronger PF activity with CF activity, which are generated by both Ca^{2+} entry through VGCCs and IP3-mediated Ca^{2+} release. Thus, both the Ca^{2+} release and Ca^{2+} influx coincidence detection mechanisms exhibit the greatest degree of supralinearity when PF activity precedes CF activity, although simultaneous PF/CF activity also produces supralinear Ca^{2+} signals [21, 66]. While the mechanisms underlying this timing dependence are not known, one possibility is that slow mGluR-mediated production of IP3 following PF activity and fast activation of Ca^{2+} entry with depolarization results in maximal IP3-mediated Ca^{2+} release when PF activity precedes CF activity. Mechanisms other than Ca^{2+} signaling also could contribute to the timing requirements of LTD.

Kinetics of LTD One of the most remarkable properties of LTD is that although the Ca^{2+} signals produced by synaptic activity last for a few minutes or less [5, 20, 21, 24, 88], the synaptic modifications induced by these Ca^{2+} signals typically occur several to tens of minutes later and last for hours or longer [89–91]. While many Ca^{2+}-dependent targets have been implicated in the expression of LTD, recent studies have begun to elucidate how the transient Ca^{2+} signals that trigger LTD are propagated in time via self-sustaining downstream signaling pathways that cause the gradual development of LTD.

There is general agreement that protein kinase C (PKC) serves as a central target of Ca^{2+} action. PKC activity in Purkinje cells is both necessary and sufficient for the induction of LTD. LTD is prevented by PKC inhibitors [92, 93], including introduction of a PKC inhibitory peptide selectively in Purkinje cells [94, 95]. Conversely, activators of PKC mimic LTD and occlude LTD induced by synaptic activity [92, 96, 97]. PKC works, at least in part, by phosphorylating Ser880 on the GluA2 subunit of the AMPAR [97, 98], which leads to internalization of AMPARs via clathrin-dependent endocytosis [9, 26].

PKC is activated during the synaptic activity that induces LTD and remains persistently active for many minutes afterwards. This persistent activation of PKC is required to produce a gradual reduction in synaptic transmission during the expression of LTD [99], arguing that it is the persistence of PKC activity that propagates Ca^{2+} signaling in time to produce LTD. Computational modeling has hypothesized that the persistent activation of PKC is produced by a Ca^{2+}-triggered positive feedback loop. This loop is proposed to include PKC and two additional enzymes—mitogen-activated protein kinase (MAPK) and phospholipase A2 (PLA2)—that mutually activate each other to produce a sustained activation of PKC [100, 101]. Experimental results have demonstrated that PKC, MAPK, and PLA2 each are required for LTD [85, 92–95, 102, 103]. Ca^{2+} uncaging experiments demonstrate that each of these enzymes works downstream of Ca^{2+} signaling [99]. Further tests of this hypothesis establish that PKC works upstream of MAPK and that MAPK also works upstream of PKC; moreover, this reciprocal activation is required to express LTD, as predicted by the hypothesis. Finally, timed application of PKC and PLA2 inhibitors shows that inhibiting these enzymes as late as 20–30 min after synaptic activity reduces the magnitude of synaptic depression (Fig. 4d), thereby demonstrating that sustained activity of PKC (and PLA2) is required to establish the early phase of LTD. It has recently been shown that the positive feedback loop also includes lipid signaling related to the PLA2-cyclooxygenase signaling pathway [104]. In summary, it appears that Ca^{2+} triggers a positive feedback loop, which sustains PKC activity for at least 20–30 min following LTD induction. This positive feedback loop effectively serves as a temporal integrator that transduces transient Ca^{2+} signals into long-lasting changes in synaptic strength. Indeed, this positive feedback loop is thought to be responsible for the "leaky integrator" properties of LTD induction described above.

Other Ca^{2+} Signaling Pathways Implicated in LTD

While there is increasing agreement that the positive feedback signaling loop serves as a Ca^{2+} target during induction of LTD, there are several additional molecules that have been implicated in LTD, yet do not directly fit into this mechanism. For example, both the α and β isoforms of another well-known Ca^{2+}-dependent protein kinase, CaMKII, have been shown to be required for LTD [105, 106]. Although it is not clear how CaMKII interacts with the PKC pathway to induce or express LTD, it has been suggested that CaMKII acts by competing with a Ca^{2+}-sensitive protein phosphatase triggering LTP [106]. Alternatively, it is possible that CaMKII inhibition indirectly blocks LTD by impairing the activity of the myosin Va

motor protein that is required for transporting endoplasmic reticulum and IP3 receptors into dendritic spines and, thus, for producing the local Ca^{2+} signal required for LTD induction [25, 107]. The positive feedback loop model also does not fully account for the actions of NO [78, 81–85]. Although NO signals are superficially independent from Ca^{2+} signals, NO is shown to cooperate with Ca^{2+} to induce LTD [30] probably by lowering the $[Ca^{2+}]_i$ required to induce LTD and by enhancing IP3-dependent Ca^{2+} release in Purkinje cells [86]. Endocannabinoids are also required for LTD and are released from Purkinje cells by the Ca^{2+} signals that trigger LTD [108, 109]. However, it is not yet clear how endocannabinoids acting on presynaptic endocannabinoid receptors can contribute to postsynaptic LTD induction. Furthermore, the positive feedback model does not account for experimental observations suggesting roles for NMDAR, GluD2, and the neuronal pentraxin receptor in LTD [48, 110, 111]. Deciphering the relationships between the Ca^{2+} signals that trigger LTD and the numerous signaling pathways that regulate LTD poses an exciting challenge for future investigation.

Summary and Conclusions

A rich postsynaptic Ca^{2+} signaling network underlies the induction of LTD at the synapse between PFs and Purkinje cells. This signaling network involves both IP3-mediated release of Ca^{2+} from intracellular stores, primarily generated by PF activity, and the influx of Ca^{2+} through VGCCs, primarily resulting from CF activity. The synergistic, supralinear interaction between these two postsynaptic signaling pathways is required for LTD and is sufficient to explain many of the properties of LTD. Thus, our understanding of the Ca^{2+} signaling pathways involved in LTD is at a satisfying stage rivaling that of other forms of long-lasting synaptic plasticity.

It is important to emphasize that our review has focused on the role of Ca^{2+} in triggering the early phase of LTD expression (early LTD) that depends upon posttranslational modifications. Studies in cultured Purkinje cells indicate that there is also a prolonged phase of LTD maintenance (late LTD) that requires gene transcription and the synthesis of new proteins [89, 103]. Although it is not yet clear whether this phase is found in vivo or in slice preparations [112], such a requirement for transcription and translation is established for maintenance of other forms of long-term synaptic plasticity and is thought to be a mechanism for the consolidation of long-term memories. Thus, it is likely that there is a late phase of LTD that requires gene transcription and translation, and the degree of overlap between the signaling pathways that trigger early LTD and late LTD will need to be determined. It is clear that late LTD, like early LTD, is triggered by an increase in $[Ca^{2+}]_i$ because LTD induced by photolysis of caged Ca^{2+} is sufficient to induce an apparent late phase of LTD [26]. This suggests that some of the Ca^{2+} targets required for LTD directly or indirectly activate transcription and translation. One attractive Ca^{2+} target is the cAMP response element-binding protein (CREB), which is required for late LTD [113]. While regulating gene expression via the CREB pathway is an attractive proposal for maintaining the long duration of LTD, much more work is needed to establish the mechanisms that allow transient postsynaptic Ca^{2+} signals to trigger the late phase of LTD.

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