44 Long-Term Depression at Parallel Fiber–Purkinje Cell Synapses

Michisuke Yuzaki

Abstract

Long-term depression (LTD) at parallel fiber (PF)–Purkinje cell (PC) synapses plays an important role in cerebellar oculomotor control and classical conditioning. Climbing fiber inputs represent an error signal to generate specific temporal and spatial Ca^{2+} dynamics at PC spines leading to endocytosis of postsynaptic AMPA receptors at PF synapses during LTD induction.

Keywords

Albus · Climbing fiber · LTD · Long-term depression Marr · Motor learning · Nitric oxide · Parallel fiber

44.1 Introduction

According to the Marr–Albus–Ito theory, long-term depression (LTD) at parallel fiber (PF)–Purkinje cell (PC) synapses serves as supervised learning machinery underlying cerebellum-dependent motor learning (Ito et al. 2014). Climbing fiber (CF) inputs to PCs represent error signals to trigger endocytosis of postsynaptic AMPA receptors leading to LTD at PF–PC synapses. Motor learning is impaired in animals treated with various pharmacological reagents or genetically engineered to modify signaling cascades involved in LTD at PF-PC synapses (Yuzaki 2013). In recent years, however, many different types of synapses, including PF to molecular-layer interneuron (MLI) synapses, CF–PC synapses, and mossy fiber to granule cell synapses, have been shown to be plastic and contribute to cerebellum-dependent learning (Gao et al. 2012). Thus, many synaptic sites at which a variety of plastic changes can modulate learning in a similar manner (Edelman and Gally 2001). However, PF–PC synapses outnumber other types of synapses at least by a factor of 50, indicating the larger capacity of learning (Kawato et al. 2021). Indeed, the number of postsynaptic AMPA receptors at PF–PC synapses is more variable than that at CF–PC and PF–MLI synapses, indicating that plastic changes mainly occur at PF–PC synapses in vivo (Masugi-Tokita et al. 2007). It was controversial whether long-term potentiation (LTP) (Gutierrez-Castellanos et al. 2017; Schonewille et al. 2010) or LTD mediates cerebellum-dependent motor learning. Here, we summarize recent findings supporting a crucial role of LTD in the oculomotor control and molecular mechanisms underlying it.

M. Yuzaki (✉)
Department of Physiology, Keio University School of Medicine, Tokyo, Japan
e-mail: myuzaki@keio.jp

44.2 LTD-Dependent Endocytosis of AMPA Receptors

Similar to LTD in many brain regions, LTD at PF–PC synapses is mediated by clathrin-dependent endocytosis of postsynaptic AMPA receptors. A key event triggering this is phosphorylation of the GluA2 subunit of AMPA receptors at serine 880 residue (GluA2-S880) by protein kinase Cα (PKC; Fig. 44.1b) (Matsuda et al. 2000; Xia et al. 2000). AMPA receptors are stabilized at synapses via their binding with glutamate-interacting protein (GRIP). Phosphorylation at S880 drastically reduces GluA2's affinity for GRIP, but not for protein interacting with C kinase 1 (PICK1), another anchoring protein that promotes AMPA receptor endocytosis. Therefore, inhibiting GluA2 interactions with GRIP or PICK1 impairs LTD induction (Matsuda et al. 2000; Steinberg et al. 2006; Xia et al. 2000).

PF inputs activate postsynaptic metabotropic glutamate receptor subtype 1 (mGluR1), leading to phospholipase C activation and production of inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol. IP_3 then induces Ca^{2+} release through IP_3 receptors, while diacylglycerol stimulates PKCα in coordination with Ca^{2+}. On the other hand, CF inputs gen-

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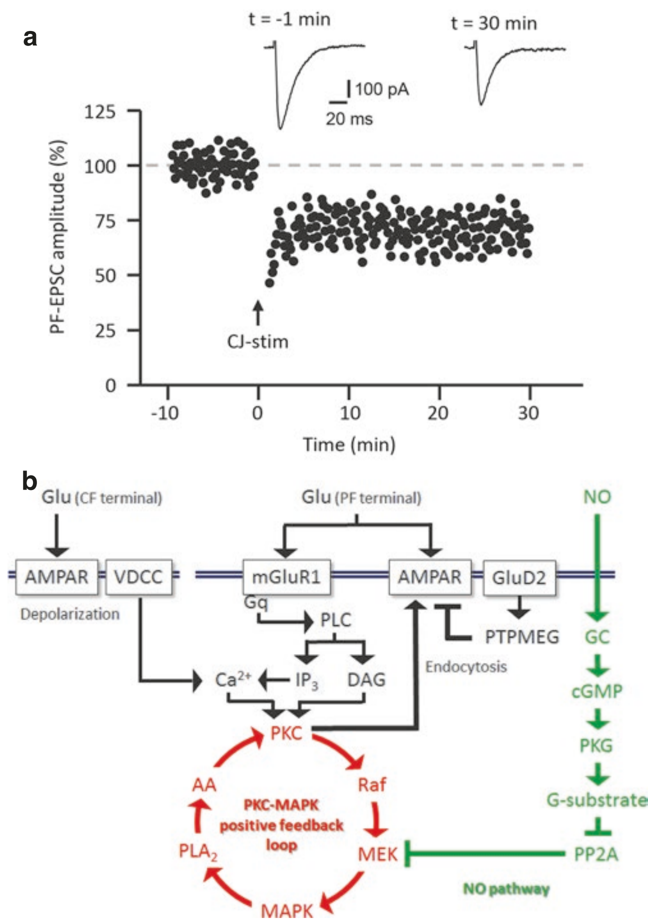

Fig. 44.1 LTD and its signaling cascades. (**a**) A typical diagram of LTD in a whole-cell patch-clamp recording. Excitatory postsynaptic currents (EPSCs; insets) of a Purkinje cell were elicited by stimulating parallel fibers (PFs) and their amplitudes are plotted against time. Conjunctive stimulations (CJ-stim) of PFs and a climbing fiber induced enduring reduction of PF-EPSCs. (**b**) A positive-feedback model together with NO and GluD2 pathways for LTD induction

erate large depolarizations triggering Ca^{2+} influx through voltage-gated Ca^{2+} channels. Interestingly, combined PF and CF activity is detected by the supralinear summation of signals coming from two Ca^{2+} sources: IP_3-mediated Ca^{2+} release from intracellular stores and Ca^{2+} influx through Ca^{2+} channels. Temporal and spatial Ca^{2+} dynamics in dendritic spines can explain several features of LTD, such as synapse specificity and dependence on the timing of PF and CF activation (Finch et al. 2012). Moreover, concurrent activation of PF and CF inputs induces a sustained (>20 min) increases in PKC activity by the positive-feedback cycle, consisting of mitogen-activated protein kinase (MAPK), phospholipase A2 (PLA2), and their related molecules (Fig. 44.1b) (Tanaka and Augustine 2008).

AMPA receptors freed from anchoring proteins diffuse into the endocytic zone located at perisynaptic sites. There, AMPA receptors first associate with clathrin adaptor protein complex-2 (AP-2). AP-2 binds to dephosphorylated forms of transmembrane AMPA receptor regulatory proteins (TARPs) (Matsuda et al. 2013; Nomura et al. 2012). Eventually, TARPs change their binding partner to AP-3, which regulates the late endosomal and lysosomal trafficking of AMPA receptors, a step necessary for the late phase of hippocampal (Matsuda et al. 2013) as well as cerebellar (Kim et al. 2017) LTD. Thus, LTD consists of two Ca^{2+}-dependent steps: phosphorylation of GluA2 to dissociate from GRIP at postsynaptic sites, and dephosphorylation of TARPs to recruit AP-2 at the endocytic zone.

Aside from activity-dependent changes, morphological changes are also observed at PF–PC synapses after motor learning in vivo (Aziz et al. 2014). In cultured PCs, LTD reportedly transits into a late phase (> ~60 min), which requires transcription of mRNAs, such as Arc (Smith-Hicks et al. 2010). However, whether and how late-phase LTD is induced in vivo remains elusive.

44.3 Optogenetic Control of Cerebellar LTD

The controversy about the role of LTD in oculomotor learning is partly caused by various LTD induction protocols used in in vitro slice preparations (Suvrathan et al. 2016). In addition, compensatory mechanisms could modify synaptic plasticity in the remaining circuits and affect motor learning in genetically engineered mice (Gao et al. 2012; Ito et al. 2014). To circumvent these problems, an optogenetic tool, termed PhotonSABER, has been developed to inhibit postsynaptic AMPA receptor endocytosis, the final common step of LTD, by neutralizing the lumen of early endosomes with a photosensitive proton pump (Kakegawa et al. 2018) (Fig. 44.2a). Light stimulation acutely and reversibly inhibited LTD in acute slice preparations, in which PhotonSABER was specifically expressed in PCs, without affecting basal synaptic transmission or other forms of synaptic plasticity, such as LTP. Furthermore, fiberoptic illumination to PCs expressing PhotonSABER in vivo inhibited adaptation of the horizontal optokinetic response (Fig. 44.2b) and vestibulo-ocular reflex. Importantly, analyses using quantitative and highly sensitive SDS-digested freeze-fracture replica labeling (SDS-FRL) revealed that the decrease in the number of postsynaptic AMPA receptors in the flocculus after the adaptation of the optokinetic response (Wang et al. 2014b) was completely inhibited by fiberoptic illumination to PCs expressing PhotonSABER (Kakegawa et al. 2018) (Fig. 44.2c). These

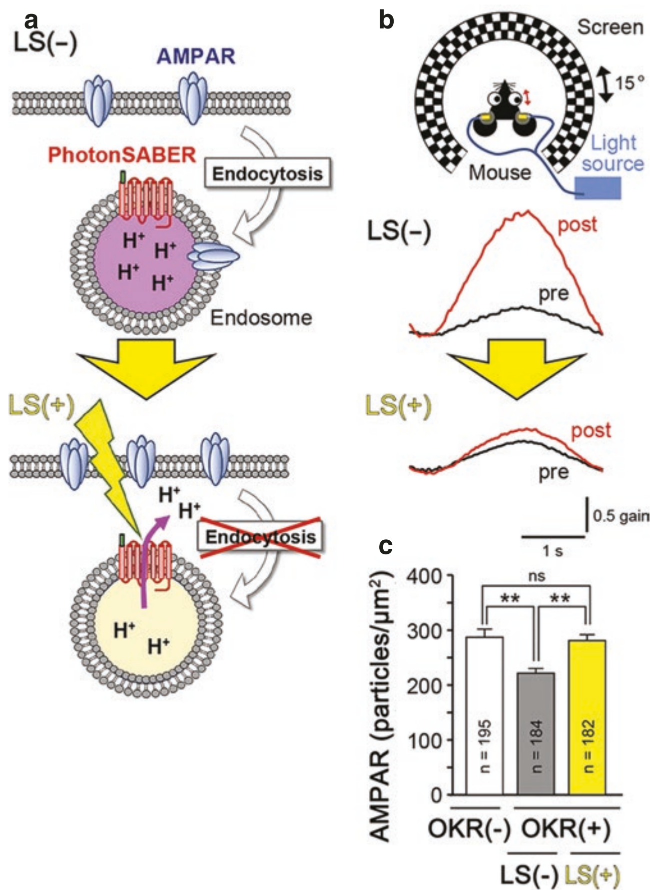

Fig. 44.2 PhotonSABER regulates AMPA receptor endocytosis and oculomotor learning. (**a**) Schematic drawing of PhotonSABER. PhotonSABER regulates the endocytosis of AMPARs by de-acidifying the endosomal lumen through light-stimulated H^+ pump activities. LS, light stimulation. (**b**) PhotonSABER inhibits the optokinetic response (OKR). Without light stimulation (LS(−)), the eye movements increase after 60-min exposures to sinusoidal oscillation (15°) of a checked-pattern screen (black traces, pre-exposure; red traces, post-exposure) in front of mice. Fiberoptic illumination (LS(+)) to the bilateral flocculi inhibits OKR adaptation. (**c**) PhotonSABER inhibits decrease in AMPA receptors following OKR. The number of AMPA receptors on freeze-fracture replicas from the flocculus decrease after OKR. The decrease is inhibited by fiberoptic illumination to the flocculi during OKR

results indicate a crucial role of LTD in the flocculus in mediating the oculomotor learning in vivo.

44.4 Unique Features of Cerebellar LTD

There are several unique features of cerebellar LTD. First, backpropagation of action potentials to dendrites, which releases voltage-dependent block of NMDA receptors by Mg^{2+}, serves as a coincidence detector for LTP/LTD induction in most neuronal circuits in the cortex, striatum, and hippocampus. In contrast, action potentials do not backpropagate to PC dendrites because of their long electrotonic distance (Vetter et al. 2001) and the low density of Na^+ channels (Stuart and Hausser 1994). In addition, functional NMDA receptors are not highly expressed in Purkinje cells in adult rodents (Perkel et al. 1990). Indeed, LTD is normally induced in acute cerebellar slices prepared from PC-specific NMDA receptor knockout mice (Kono et al. 2019). Instead of Ca^{2+} influx through NMDA receptors, the supralinear summation of Ca^{2+} signals from PF-evoked Ca^{2+} release from IP3 receptors and CF-evoked Ca^{2+} influx through voltage-gated Ca^{2+} channels serves as a coincidence detector for LTP/LTD in cerebellar PCs. Thus, compared to the detection of coincidence by NMDA receptors with a temporal window of ~10 ms (Markram et al. 1997) in hippocampalal neurons, CF-evoked Ca^{2+} changes are very slow, reflecting activation of mGluR1, production of IP3 and Ca^{2+} release from IP3 receptors in PCs. Therefore, PCs are suited to detect coincidence of PF/CF activities in a wider time window. For example, the window is expected to be ~30 ms for controlling ocular-following responses in the paraflocculus, and longer than 100 ms for regulating reaching tasks in the cerebellar hemisphere (Suvrathan et al. 2016). It remains unclear how such time windows matching the motor-sensory time delay associated with different movements are achieved by PCs located in different cerebellar regions.

Instead of NMDA receptors, cerebellar LTD requires the δ2 glutamate receptor (GluD2). GluD2 is highly and predominantly expressed in PC dendrites. While GluD2's channel activities are not required for LTD induction, its intracellular C-terminal region is indispensable (Kakegawa et al. 2008; Kohda et al. 2007) since it binds to PTPMEG, a protein tyrosine phosphatase that dephosphorylates a tyrosine residue (Y876) of GluA2. Interestingly, prior phosphorylation of Y876 hindered subsequent phosphorylation at S880 by PKC (Kohda et al. 2013). Thus, in *GluD2*-null or *PTPMEG*-null PCs, GluA2-Y876 is highly phosphorylated and LTD-inducing stimuli fail to phosphorylate S880 (Fig. 44.3). Therefore, GluD2 controls LTD induction by regulating interactions between the two phosphorylation sites of GluA2. Cbln1, a C1q family protein released from PFs, binds to the extracellular N-terminal region of GluD2 (Matsuda et al. 2010). Although it remains unclear why LTD is impaired in *Cbln1*-null mice (Hirai et al. 2005), PTPMEG signaling at the C-terminus may be regulated by binding of Cbln1 at the N-terminus of GluD2.

Nitric oxide (NO), which is produced by NO synthase (NOS) following Ca^{2+} influx through NMDA receptors, serves as a retrograde messenger to induce long-term potentiation (LTP) in hippocampal neurons (Padamsey and Emptage 2014). LTD is impaired in mice genetically lacking neuronal NOS (Lev-Ram et al. 1997). LTD and oculomotor learning are impaired in MLI/PC-specific, but not granule cell- or PC-specific NMDA receptor knockout

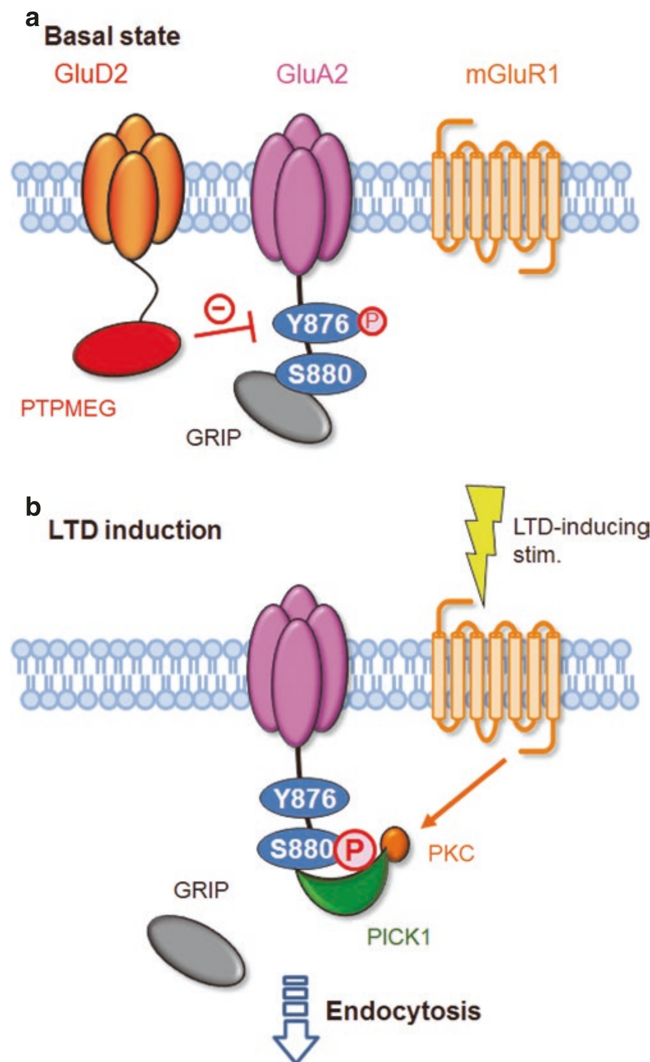

Fig. 44.3 A proposed model of how GluD2 regulates cerebellar LTD. (**a**) Basal state. GluD2 maintains low phosphorylation levels at Y876 of the GluA2 subunit of AMPA receptors via PTPMEG, a protein tyrosine phosphatase. (**b**) LTD induction. LTD-inducing stimuli further dephosphorylate this tyrosine residue. Y876 dephosphorylation allows S880 phosphorylation by protein kinase C, which leads to the replacement of GRIP, a membrane anchoring protein, with PICK1 to allow AMPA receptor endocytosis

mice, indicating an important role of NMDA receptors in MLIs (Kono et al. 2019). Since application of an NO donor restored LTD in MLI/PC-specific NMDA receptor knockout cerebellar slices, NO is likely produced by activation of NMDA receptors in MLIs during LTD. NO upregulates the MAPK pathway in the positive-feedback loop of LTD induction (Fig. 44.1b). NO is also necessary for postsynaptic LTP induced by stimulation of PFs at 1 Hz (Kakegawa and Yuzaki 2005; Lev-Ram et al. 2002). However, this form of LTP is intact in NMDA receptor knockout mice (Kono et al. 2019). Instead, 1 Hz PF stimulation activates cannabinoid receptor 1 to produce NO in PFs (Wang et al. 2014a). Thus, NO may not be directly involved in LTD induction, but rather plays a role in other aspects, such as the spread of plasticity across synapses.

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