

Systems Biology Perspectives on Cerebellar Long-Term Depression

Hideaki Ogasawara^{a,b} Tomokazu Doi^c Mitsuo Kawato^b

^aNational Institute of Information and Communications Technology, ^bATR Computational Neuroscience Laboratories, Kyoto, and ^cOsaka Bioscience Institute, Osaka, Japan

Key Words

Cerebellum · Long-term depression · Systems biology · Simulation · Intracellular signaling cascade · Protein kinase M ζ

Abstract

Long-term depression (LTD) at parallel fiber-Purkinje cell (PF-PC) synapses is thought to be the cellular correlate of cerebellar associative learning. The molecular processes are, in brief, phosphorylation of AMPA-type glutamate receptors (AMPA-Rs) and their subsequent removal from the surface of the PF-PC synapse. In order to elucidate the fundamental mechanisms for cerebellar LTD and further the understanding of its computational role, we have investigated its systems biology and proposed the following hypotheses, some of which have already been experimentally verified: (1) due to the mitogen-activated protein kinase (MAPK)-protein kinase C (PKC) positive feedback loop, phosphorylation of AMPARs is an all-or-none event; (2) the inositol 1,4,5-triphosphate receptor detects concurrent PF and climbing fiber inputs, forming the cellular basis for associative learning, and (3) the local concentration of nitric oxide in the PC dendrite reflects the relevance of a given context, enabling context-dependent selection of learning modules within the cerebellum. In this review, we first introduce theoretical studies on cerebellar LTD, mainly focusing on our own published work, followed by a discussion of the effects of stochasticity, localization, diffusion, and scaffolding. Neu-

rons embody two features that are apparently contradictory, yet necessary for synaptic memory: stability and plasticity. We will also present models for explaining how neurons solve this dilemma. In the final section, we propose a conceptual model in which a cascade of excitable dynamics with different time scales, i.e., Ca²⁺-induced Ca²⁺ release, the MAPK-PKC positive feedback loop, and protein kinase M ζ (PKM ζ)-induced PKM ζ synthesis, provides a mechanism for stable memory that is still amenable to modifications.

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Introduction

The cerebellum is important in motor coordination, adaptation, and learning, as well as in language, cognition, and many other aspects of life [reviewed in 1–7]. It is thought to be a specialized organ for supervised learning (also known as associative learning, through which each input signal is specifically associated with a desired output) [8, 9]. The main neurons and wirings in the cerebellar cortex include Purkinje cells (PCs), parallel fibers (PFs), and climbing fibers (CFs). PCs provide the sole output from the cortex, and each PC receives two types of excitatory inputs: one from hundreds of thousands of PFs and the other from a single CF. The Marr-Albus-Ito theory [10–12] states that their neuronal circuit underlies associative learning. In the theory, PFs provide a sensorimotor context to PCs, while CFs carry teacher signals

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1424–862X/08/0164–0300\$24.50/0

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Dr. Hideaki Ogasawara
ATR Computational Neuroscience Laboratories
2-2-2, Hikaridai, Seikacho, Kyoto 619-0288 (Japan)
Tel. +81 774 95 2688, Fax +81 774 95 1259
E-Mail ogahide@atr.jp

that modify PF-PC synapses in an associative manner. After a long investigation, the PF-PC synapse was experimentally demonstrated to be plastic; its transmission efficacy was depressed when the CF and PF were repetitively and synchronously activated (cerebellar long-term depression or LTD) [13]. Subsequent *in vitro* studies have revealed that LTD is regulated by $[Ca^{2+}]$ [14–16] and that $[Ca^{2+}]$ elevation and PF-PC LTD are most prominent when PF stimuli precede CF stimuli by 50–250 ms [17, 18] (N.B.: In this article, [substance] stands for the concentration of the substance). In behaving animals, CF inputs are delayed ~100 ms with respect to PF inputs [3–5, 11], due to the definite sequence of events during motor execution (more specifically, first, PCs receive PF inputs, motor commands are given and followed by a motion, the feedback control circuit generates feedback motor commands, which are finally transmitted through CFs to the PCs as error signals [3]). The similarity between the *in vitro* time window size and *in vivo* PF-CF delay length strongly supports the Marr-Albus-Ito theory that PF-PC plasticity is the cellular process of cerebellar learning [10–12], although some controversies exist [19, 20].

The molecular mechanism of PF-PC LTD involves the internalization of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors (AMPA receptors), which occurs when activated protein kinase C (PKC) phosphorylates the receptor GluR2 subunit. Essential molecules for LTD include mitogen-activated protein kinase (MAPK), MAPK kinase (MAPKK), MAPKK kinase (MAPKKK), PKC, phospholipase A2 (PLA2) and C (PLC), arachidonic acid (AA), inositol 1,4,5-triphosphate (IP_3), and many others [reviewed in 4, 7, 21–26].

Postsynaptic long-term potentiation (LTP) reverses PF-PC LTD [27–29]. Without such reversal mechanisms, LTD would eventually reach saturation, preventing the occurrence of further learning events. A presynaptically synthesized messenger nitric oxide (NO) is a crucial ‘gatekeeper’ [30] for cerebellar plasticity; LTD and LTP are induced only in the presence of NO, and its deprivation prevents both LTD and LTP [27, 31–36, reviewed in 7, 37]. The direction of gain change is $[Ca^{2+}]$ -dependent, with a high threshold for LTD and a low threshold for LTP [25, 29]. Cerebellar adaptation has also been shown to be NO-dependent [38–40].

The number of molecular species known to engage in PF-PC plasticity is still increasing; Ca^{2+} /calmodulin-dependent protein kinases II (CaMKII) [41] and IV (CaMKIV) [42, 43], δ_2 receptors [44, 45], and endocannabinoid [46] are among the growing list. However, an

extension of this list of implicated molecules does not necessarily further the fundamental understanding of plasticity mechanisms. As has been claimed [47], it is important to determine the minimal set of ‘mediators’ and their interactions that are essential in the memory formation processes.

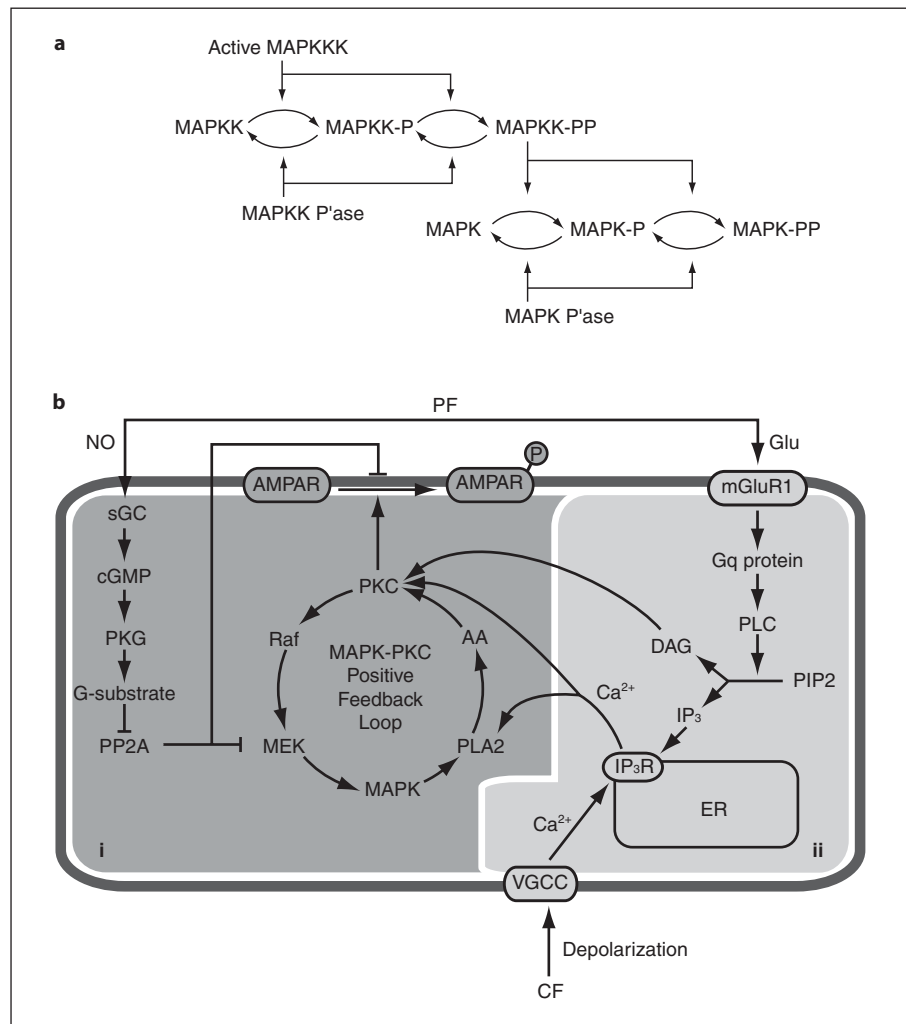
To elucidate the key pathways of LTD and their computational roles in cerebellar learning, we have studied the systems biology of PF-PC synaptic plasticity and proposed the following hypotheses: (1) MAPK and PKC activate each other, generating a positive feedback loop, and because of this loop, phosphorylation of AMPARs is an all-or-none event [48]; (2) the IP_3 receptor (IP_3R) is capable of detecting conjunctive PF and CF inputs [49], which is a necessary feature for cerebellar associative learning; (3) NO reflects the relevance of a given context and enables context-dependent selection of learning modules in the cerebellum [30]. In this article, we first review the theoretical studies on cerebellar LTD, mainly focusing on our own published work, followed by a discussion of the possible effects of stochasticity, localization, diffusion, and scaffolding on synaptic transmission and plasticity. The brain is capable of learning new things while maintaining old memory. At the cellular level, this means that synapses must be both stable and plastic at the same time. How do they attain these contradictory characteristics [50]? We will present models for explaining how neurons solve the ‘stability versus plasticity dilemma’. In the final section, we propose a conceptual model in which cerebellar LTD is induced and maintained by successive activation of three bistable dynamics with different time scales – Ca^{2+} -induced Ca^{2+} release, the MAPK-PKC positive feedback loop, and protein kinase M ζ (PKM ζ)-induced PKM ζ synthesis. This molecular network is predicted to make cerebellar memory stable for a very long period of time but still amenable to modifications.

Simulation Studies on Cerebellar LTD

1. The MAPK-PKC Positive Feedback Loop Is the Cerebellar LTD Switch

The mechanisms by which cells respond to transient and/or graded stimuli, and exhibit a switch-like behavior, have been drawing researchers’ interest for decades [reviewed in 51–56]. One such mechanism produces a continuous response that is more sensitive than Michaelis-Menten kinetics to changes in the stimulus amplitude; it

Fig. 1. Signaling cascades of cerebellar LTD. **a** The MAPK cascade. MAPKK-P and MAPKK-PP denote singly and doubly phosphorylated MAPKK, respectively. MAPK-P and MAPK-PP denote singly and doubly phosphorylated MAPK. P'ase denotes phosphatase. Adapted from Huang and Ferrell [67]. Copyright 1996 by the National Academy of Sciences. **b** Schematic view of signaling cascades in cerebellar LTD. Reactions surrounded in the bold gray line take place inside the dendritic spine. **i** Dark gray area = the MAPK-PKC positive feedback loop [48] and its peripherals. **ii** Light gray area = PF-CF coincidence detection mechanisms [49]. Glu = Glutamate; PKG = cGMP-dependent protein kinase; PIP2 = phosphatidylinositol bisphosphate.



is termed 'ultrasensitivity' [57], and the most familiar examples are cooperative enzymes. In other cases, a cellular system has two stable steady states and jumps from one to the other, avoiding the intermediate states. This kind of switch is termed 'bistability' [54]. A bistable system displays different stimulus-response relationships, depending on whether the system began in the ON state or the OFF state (hysteresis). Bistability is particularly important in neurobiology, since it has been implicated in the storage of cellular information.

In the 1950s, autocatalytic enzymes were predicted to have multiple stable steady states [58]. Conceptual models were proposed in the 1980s describing a biological switch formed by a kinase that is activated by itself or another kinase and inactivated by a phosphatase; these models, however, were abstract and lacked a solid molecular background [59, 60]. Progress in molecular bi-

ology has revealed that the MAPK cascade is very important in information processing of neurons and other types of cells [reviewed in 56, 61–66]. The cascade consists of MAPKKK, MAPKK, and MAPK (fig. 1a); MAPKKK activates MAPKK by dual phosphorylation, and similarly, MAPKK activates MAPK by dual phosphorylation [65]. Biochemical experiments and realistic simulations have shown that these 'two-collision mechanisms' result in ultrasensitivity of the MAPK cascade [67]. In theory, ultrasensitivity arises also when two opposing enzymes (e.g., a kinase and a phosphatase) operate at near-saturation levels (zero-order ultrasensitivity) [57]. An ultrasensitive MAPK cascade response can be converted into a true all-or-none bistable response when there is a pathway that connects the output and input of the MAPK cascade and forms a positive feedback loop [68–70; but see also 71].

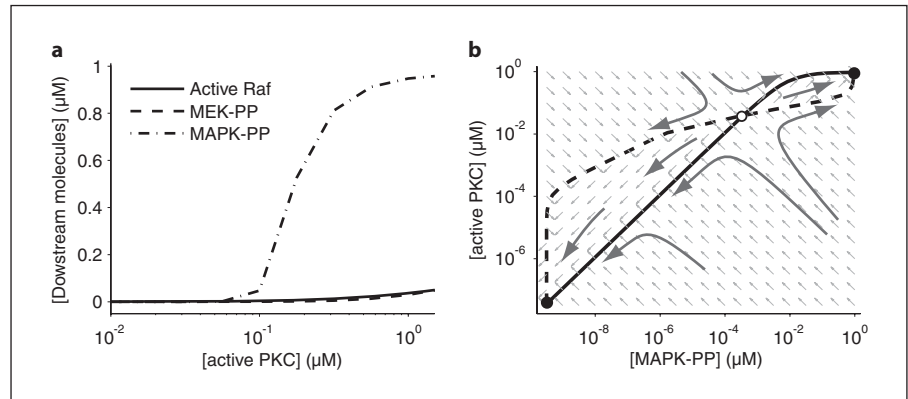


Fig. 2. Supralinearity and bistability of the MAPK-PKC positive feedback loop. **a** [Active Raf], [MEK-PP], and [MAPK-PP] plotted against various concentrations of active PKC that were kept constant throughout each simulation. [Active PP2A] was kept constant at 85 nM, and total concentrations of Raf, MEK, and MAPK were 0.5, 0.5 and 1 μM , respectively. **b** Phase plane analysis of the MAPK-PKC positive feedback loop. [Active PP2A] was kept constant at 85 nM. The solid bold line indicates the steady state [active PKC] plotted against various concentrations of MAPK-PP that

were kept constant throughout each simulation; likewise, the dashed bold line indicates the steady state [MAPK-PP] plotted against various [active PKC]. Filled circles and an open circle indicate stable steady states and an unstable steady state, respectively. Thin arrows indicate one of four directions, i.e., northeast, northwest, southwest, and southeast, which each point in the phase plane is directed towards. Schematic trajectories are superimposed (thick arrows).

Kuroda et al. [48] hypothesized that the MAPK cascade and other enzymes in the PC, e.g., PLA2 and PKC, make a positive feedback loop, which plays a pivotal role in LTD. The following is a description of the feedback loop and the peripheral pathways that they modeled (fig. 1b-i). Conjunctive PF-CF inputs result in increased $[\text{Ca}^{2+}]$ and [diacylglycerol (DAG)] in PC dendritic spines. Ca^{2+} and DAG transiently activate conventional PKC (cPKC), a PKC isoform that is sensitive to both Ca^{2+} and DAG. cPKC also activates Raf, a MAPKKK, and Raf activates MEK, a MAPKK, through dual phosphorylation. Similarly, MEK activates MAPK through dual phosphorylation. Activated MAPK, as well as Ca^{2+} , activates PLA2, resulting in the production of AA and subsequent activation of cPKC. In this way, the MAPK cascade, PLA2, AA, and cPKC form a positive feedback loop (MAPK-PKC positive feedback loop). Activated cPKC phosphorylates AMPARs, and the phosphorylated receptors are eventually removed from the postsynaptic membrane through endocytosis. Thus, the magnitude of LTD corresponds to AMPAR phosphorylation. PF firing induces presynaptic synthesis of NO, which diffuses across the synaptic cleft and activates soluble guanylyl cyclase (sGC), which, in turn, catalyzes the conversion of guanosine triphosphate to cyclic guanylyl cyclase (cGMP). cGMP activates cGMP-dependent protein kinase, which phosphorylates G-substrate. Phosphorylated G-substrate eventually inactivates

protein phosphatase 2A (PP2A), an enzyme that dephosphorylates MEK and AMPARs. The Kuroda et al. model deals only with the initial and intermediate phases of cerebellar LTD, when cPKC is essential [72], but does not consider the late phase, which is cPKC-independent [72] and requires new protein synthesis [4, 7, 21].

Simulations revealed that the initial phase of cerebellar LTD is dependent on direct activation of cPKC by Ca^{2+} and DAG, whereas the intermediate phase is mediated by activation of the MAPK-PKC positive feedback loop. The model also demonstrated an all-or-none property of AMPAR phosphorylation, within a time scale of approximately 40 min. There was a sharp threshold level of input; stimuli greater than the threshold stereotypically resulted in AMPAR phosphorylation that was persistent for more than half an hour, whereas stimuli smaller than the threshold did not. This simulation result correlates with recent experiments and hypotheses that suggest that synapses alter their strength by jumping between discrete states, rather than shifting gradually [73–75].

Previous studies [68, 69] have shown that the ultrasensitivity of the MAPK cascade is essential for the all-or-none property of the feedback loop the cascade constitutes, which the Kuroda et al. model is in accordance with. Figure 2a plots [active Raf], [doubly phosphorylated MEK (MEK-PP)], and [doubly phosphorylated MAPK

(MAPK-PP) against various concentrations of active PKC that were manipulated to remain constant throughout each simulation (data was produced for this review article; the model script can be found at <http://www.cns.atr.jp/neuroinfo/kuroda/> and runs on simulation software, GENESIS/Kinetikit [76, 77]). The cascade output, [MAPK-PP] describes a sigmoidal curve with a large Hill coefficient of 4.5 (fig. 2a), indicating pathway ultrasensitivity. As mentioned earlier, the ultrasensitivity of the MAPK cascade is a result of the two-collision mechanisms of MEK and MAPK [67]. Zero-order ultrasensitivity [57] also seems to contribute because [active Raf] and [MEK-PP] are at ranges that are much lower than the concentrations of their substrates (fig. 2a).

AMPA phosphorylation in the Kuroda et al. model is not permanent because the model takes into consideration the degradation of activated cPKC and recovery of PP2A from NO inactivation. To elucidate the dynamics of the MAPK-PKC positive feedback loop, these slow processes were eliminated from the model to guarantee the stability of the active state, and a phase plane analysis was performed for this review article (fig. 2b). Phase plane profiles are curves of one dependent variable against another and are used to determine whether a model has one or more steady states [78, also refer to 79 for another method that is useful for analyzing positive feedback systems consisting of more than two variables]. Nullclines in a phase plane indicate the values of a pair of variables, in which one of the variables is constant; the points of intersection are steady states. Two nullclines are shown in figure 2b; one is [active cPKC] against [MAPK-PP] (solid bold line), and the other is [MAPK-PP] against [active cPKC] (dashed bold line). Schematic trajectories are superimposed (thick arrows). The figure indicates that the MAPK-PKC positive feedback loop is a bistable system, having two stable steady states (filled circle) and an unstable saddle point (open circle). It also demonstrates that the supralinear response of [MAPK-PP] against [active PKC] is critical for the bistability of the system because two curves would not intersect at more than two points if the response of MAPK-PP to active PKC followed a more gradual kinetics, such as Michaelis-Menten kinetics.

Tanaka et al. [72, 80] performed a series of slice experiments and verified the theoretical study. First, they demonstrated that MAPK acts downstream of PKC; a PKC inhibitor prevented MAPK activation and LTD that were induced by an LTD-induction protocol. Second, a MAPK inhibitor blocked the translocation of PKC, which is an indication of kinase activity. These findings indicate

the existence of essential pathways through which MAPK and cPKC activate each other, supporting the hypothesis that MAPK and cPKC form a positive feedback loop that is pivotal for cerebellar LTD [48].

By locally photolyzing caged calcium and using confocal imaging [81], Tanaka et al. subsequently demonstrated that elevation of $[Ca^{2+}]$ alone is sufficient for the induction of cerebellar LTD, and they quantified the relationship between $[Ca^{2+}]$ and LTD for the first time. LTD was induced stereotypically by $[Ca^{2+}]$ elevations of substantial peak and duration, while small or short-term elevations in $[Ca^{2+}]$ resulted in almost no LTD (fig. 3a). The sigmoidal relationship between peak $[Ca^{2+}]$ and the amount of LTD (each curve in fig. 3a) was so supralinear that it could be described by the Hill equation with a large Hill coefficient of 5 (fig. 3a). Even though LTD was a supralinear function of $[Ca^{2+}]$, the all-or-none property, which Kuroda et al. [48] had predicted, was not observed. It should be noted that Tanaka et al. measured average LTD of dozens of nearby synapses, not LTD of single synapses. The discrepancy between the experiments and simulations is reminiscent of previous experiments demonstrating that individual synaptic plasticity is discrete and heterogeneous, while these synapses present graded plasticity as a whole [75]. By modifying the Kuroda et al. model for a special form of LTD that was induced solely by Ca^{2+} and incorporating heterogeneity and noise into the model, the authors accurately simulated experimental measurements (fig. 3b) and confirmed the usefulness of the model in prediction and explanation of future experiments.

In order to verify that the MAPK-PKC positive feedback loop (fig. 1b) is the underlying mechanism for supralinearity of LTD, they examined the relationship between $[Ca^{2+}]$ and LTD subsequent to pharmacological prevention of AA production [80]. Treatment of cerebellar slices with a PLA2 inhibitor resulted in a stimulus-response curve that was much more gradual with a greatly reduced Hill coefficient (fig. 3c). This result was in agreement (qualitatively and quantitatively) with the corresponding simulation in which AA activation of cPKC was blocked (fig. 3d). The finding that positive feedback loop disruption results in the loss of supralinearity supports the hypothesis that the loop has a vital role in cerebellar LTD [48]. In addition, the results are in accordance with previous experiments and theoretical studies on the dynamics of positive feedback loops in biological contexts [53, 68, 69].

Both the experiments and simulations revealed that the amount of required $[Ca^{2+}]$ for LTD induction was a

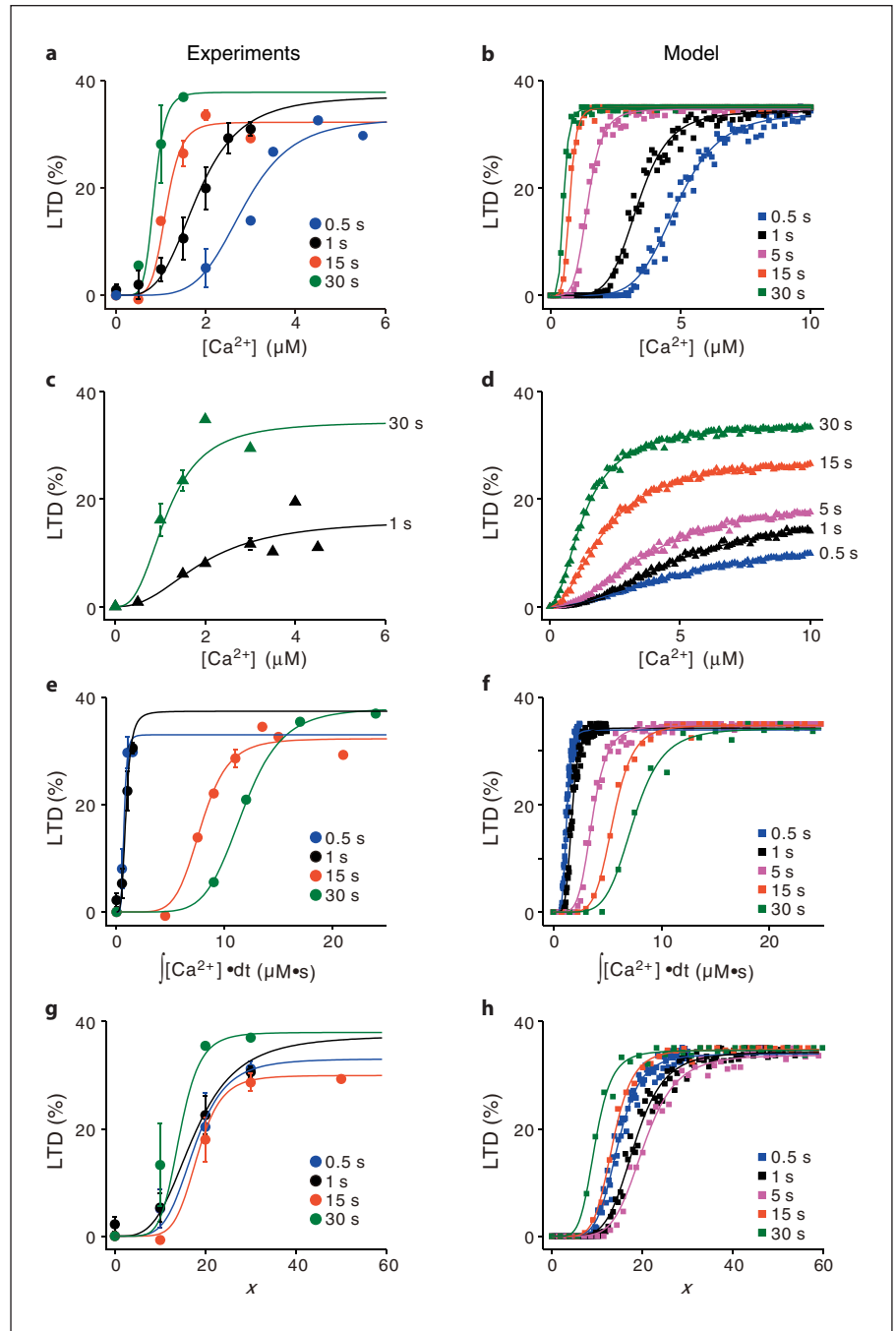


Fig. 3. Integration of $[Ca^{2+}]$ during LTD. Results of experiments (**a, c, e, g**) and simulations (**b, d, f, h**) are shown side by side. **a, b** Relationship between peak $[Ca^{2+}]$ and LTD for different durations of $[Ca^{2+}]$ elevation. **c, d** Relationship between peak $[Ca^{2+}]$ and LTD for different durations of $[Ca^{2+}]$ elevation in the presence of a PLA2 inhibitor. **e, f** Relationship between integrated amount of $[Ca^{2+}]$ and LTD. Data from the same experiments and simulations shown in panels **a** and **b**, respectively. **g, h** Data from panels **a** and **b** transformed by calculating x from equation 2. Smooth curves indicate fits of the Hill equation. Error bars in panels **a, c, e, and g** indicate standard errors of measurement. This figure is reproduced from Tanaka et al. [80] with permission from Elsevier Inc.

time-dependent variable. The longer the duration of a Ca^{2+} stimulus was, the lower the necessary $[Ca^{2+}]$ peak was (fig. 3a, b), suggesting that the LTD induction mechanism integrates calcium signals over time. However, when the amount of LTD was plotted against the time integral of $[Ca^{2+}]$, the integrated amount of required $[Ca^{2+}]$ for LTD increased over time instead of being time-

independent (fig. 3e, f), which suggests that the time integration process in LTD is somewhat leaky. Such a leaky integration of $[Ca^{2+}]$ can be described mathematically as

$$\tau \frac{dx}{dt} = -x + a[Ca^{2+}](t) \quad (1)$$

where a is a scaling factor, τ is the time constant of the integration, and x is the amount of downstream signal that transduces Ca^{2+} into LTD. In special cases where $[\text{Ca}^{2+}]$ is elevated in a ramp-like fashion as in Tanaka et al. [80], the solution of equation 1 is

$$x(t) = ak\tau \exp\left(-\frac{t}{\tau}\right) + ak(t - \tau) \quad (2)$$

where k is the rate of increasing $[\text{Ca}^{2+}]$ (peak $[\text{Ca}^{2+}]$ divided by the uncaging duration, t). After these parameters were paired to the experimental results (a and τ were $18.7\%/\mu\text{M}$ and 0.56 s, respectively), the amount of LTD was plotted against x in figure 3g and h. All the curves that were obtained at various durations of Ca^{2+} stimuli overlapped each other. The loss of time-dependence seen in figure 3a, b, e, and f verifies equation 2 and supports the hypothesis that the signaling processes of LTD behave as a leaky integrator of $[\text{Ca}^{2+}]$.

2. Coincidence Detection of Cerebellar Inputs

PF-PC plasticity is thought to be the cellular process of cerebellar supervised learning [10–12], but what molecular mechanisms enable the synapse to associate PF inputs (desired trajectories and sensory feedback) with CF inputs (error signals [3]) within a certain time window? Candidate coincidence detectors include (a) voltage-gated calcium channels (VGCCs) [23], (b) AMPARs [23], (c) PKC [23], (d) presynaptic membrane [82], and (e) IP_3Rs [17, 23, 83]. The scenarios for the candidates a–d are as follows: (a) simultaneous activation of PFs and the CF depolarizes the dendrite and induces Ca^{2+} influx through VGCCs in the spine, (b) glutamate released from PFs may sensitize AMPARs for phosphorylation, (c) protein tyrosine kinase activated by PF inputs, and $[\text{Ca}^{2+}]$ elevated by CF inputs, cooperate to activate PKC, (d) repetitive firing of PFs activates presynaptic NMDA-type glutamate receptors and induces NO synthesis. Upon simultaneous CF activity, a retrograde messenger, endocannabinoid might enhance NO release. Unfortunately, scenarios a–c cannot explain the ~ 100 -ms PF-CF delay that is optimal for $[\text{Ca}^{2+}]$ elevation and cerebellar LTD. Since there is no evidence that endocannabinoid enhances NO signaling, the presynaptic membrane (d) is not a likely candidate, either.

Then, is the IP_3R (e) a probable coincidence detector? IP_3Rs are located in a calcium store of the dendritic spine, the endoplasmic reticulum (ER). The receptor is synergistically activated by Ca^{2+} and IP_3 , and releases a large

amount of Ca^{2+} from the ER in response, whereas excessive $[\text{Ca}^{2+}]$ is inhibitory to the receptor [84, 85]. Thus, the open probability of the IP_3R is a bell-shaped function of $[\text{Ca}^{2+}]$. On the one hand, PF firing activates PLC through the mGluR1 metabotropic glutamate receptor pathway, resulting in production of IP_3 (and DAG) from phosphatidylinositol bisphosphate (fig. 1b-ii). On the other hand, CF firing depolarizes the PC and induces influx of Ca^{2+} through VGCCs. Therefore, $[\text{IP}_3]$ and $[\text{Ca}^{2+}]$ represent PF and CF activities, respectively, within the physiological range of inputs. By sensing sequential binding of IP_3 and Ca^{2+} [17, 23, 83], the IP_3R may act as a coincidence detector that associates PF inputs with CF inputs and releases a high amount of Ca^{2+} as its output signal, leading to LTD. However, massive stimulation of a PF bundle alone [86, 87], or uncaging either caged calcium or caged IP_3 [16, 88], can also induce LTD, which raises doubts that LTD is the cellular basis of cerebellar associative learning [19, 20].

To understand the spike-timing detection mechanism of cerebellar LTD, Doi et al. [49] developed a kinetic model of Ca^{2+} within a PC dendritic spine. As shown in figure 1b-ii, the model consists mainly of mGluR1s, Gq protein, PLC in the postsynaptic density (PSD), IP_3Rs in the ER, and VGCCs in the cytoplasmic membrane. The molecular pathway (as briefly mentioned in the previous paragraph) and parameter values were based on an extensive review of the biological literature. The simulations demonstrated that the supralinear Ca^{2+} response to conjunctive PF-CF inputs is a regenerative process that is driven by the IP_3R . More specifically, $[\text{Ca}^{2+}]$ elevation opens IP_3Rs , and outflow of Ca^{2+} from the ER results in additional $[\text{Ca}^{2+}]$ elevation ($\text{Ca}^{2+} \rightarrow \text{IP}_3\text{Rs} \rightarrow \text{Ca}^{2+}$), thereby producing a positive feedback loop. In theory, $[\text{Ca}^{2+}]$ elevation may enhance the activity of PLC for IP_3 production, and IP_3 elevation may open IP_3Rs to release Ca^{2+} from the ER, creating another positive feedback loop ($\text{Ca}^{2+} \rightarrow \text{PLC} \rightarrow \text{IP}_3 \rightarrow \text{IP}_3\text{Rs} \rightarrow \text{Ca}^{2+}$). However, simulations have demonstrated that this hypothetical pathway does not form a working positive feedback loop in the PC dendritic spine and denied the role of PLC in the supralinearity of Ca^{2+} influx. The Ca^{2+} response was largest when CF input followed PF input within an interval of 0–300 ms, which is consistent with experiments [17, 18] and the associative nature of cerebellar learning [3–5, 11]. The time scale difference between a rapid electrical reaction (Ca^{2+} influx by depolarization) and a slow biochemical reaction (IP_3 production by the metabotropic pathway) is critical for this spike-timing detection mechanism. Finally, there is a sharp threshold level of $[\text{Ca}^{2+}]$ for Ca^{2+} -induced Ca^{2+} release, and the threshold is regulated by

[IP₃]. In line with previous experiments [17], simulations demonstrated that a CF input within 300 ms after a PF input induced a Ca²⁺ transient, which easily reached the threshold of [Ca²⁺] for regenerative Ca²⁺ release, because the threshold level was already lowered by the PF-mGluR1 pathway product, IP₃. In contrast, when a CF input occurred either before or too late after PF inputs, the CF-mediated elevation of [Ca²⁺] failed to reach the threshold, because the threshold had already returned to a higher level as [IP₃] decreased. The IP₃-dependent Ca²⁺ threshold is capable of explaining not only conjunctive LTD, but also LTD in non-physiological conditions. Even at the baseline [IP₃], where the Ca²⁺ threshold for regenerative Ca²⁺ release was highest, an excessive increase in [Ca²⁺] could reach the threshold and induce LTD, as seen after massive activation of a PF bundle [86, 87] or Ca²⁺ uncaging [16]. Similarly, an extremely high [IP₃], typically seen upon IP₃ uncaging [88], would result in LTD without requiring an increase in [Ca²⁺] because the baseline [Ca²⁺] is already greater than the IP₃-regulated Ca²⁺ threshold. The descriptive power of the Ca²⁺ kinetics model indicates that the model captures the essence of the molecular mechanisms of cerebellar LTD.

Hernjak et al. [89] simulated the Ca²⁺ kinetics of PC dendrites and spines with realistic parameters, similarly to Doi et al. This study was unique in that they considered one- or two-dimensional diffusion and localization of molecules in addition to biochemical processes. They showed that high density and low sensitivity of the IP₃R in the PC [90] are critical for generating and localizing Ca²⁺ spikes in a single dendritic spine. They also demonstrated that Ca²⁺ was compartmentalized in spines by their narrow neck, as predicted previously [91–93], whereas IP₃ freely diffused into the dendrite and neighboring spines.

3. The Role of NO in Context-Dependent Learning

Recent studies have shown that a set of neurons that process a certain routine, such as use of scissors, are modularly organized in the cerebellum. Modules can be switched according to a given context of behavior [94–96], so that animals can adapt to multiple environments. Little is known, however, about the biological mechanisms for context-dependent switching.

Ogasawara et al. [30] hypothesized that NO was crucial in context-dependent selection of learning modules because neighboring PF activity, which reflects the context of behavior, determines local [NO]. They combined

established simulation models of electrophysiology, calcium dynamics (fig. 1b-ii), and intracellular signaling cascades (fig. 1b-i) [48, 49, 97], and further characterized the role of NO in cerebellar learning. The simulation results revealed that LTD was regulated by NO, whose concentration depended on surrounding PF activity. When PF activity in the vicinity was low, conjunctively stimulated PF-PC synapses were incapable of undergoing LTD, because of insufficient NO concentration. When PF activity was excessive, LTD spread to neighboring synapses where [NO] was high and [Ca²⁺] was relatively low, and synaptic specificity was lost. LTD occurred in a synapse-specific manner only with moderate levels of nearby PF activity. Based on these results, the authors predicted that any movement made in any context was encoded by a small percentage of PFs because otherwise LTD would not occur at all or would be unspecific. Another hypothesis was that NO enables context-dependent selection of appropriate learning modules. An animal experiment was suggested to verify their hypotheses. For the detailed procedures, refer to the original paper [30].

Stochasticity, Localization, Diffusion, and Scaffolding

Mass-action kinetics is often a good approximation of biochemical reactions in relatively simple cells, such as *Xenopus* oocytes and undifferentiated culture cells [67, 68, 98]; however, it might not correctly describe the signaling pathways of the neuron for several reasons. First, the spine of an excitatory synapse, a key unit of neuronal information processing, is very small (~1 μm or less in diameter) [99–101] and contains only a limited number of each molecular species. For instance, the number of AMPARs in a PF-PC synapse is as small as 4–73 [102]. In such cases, stochastic fluctuations come into play, and mass-action kinetics, described in continuous equations, is no longer applicable. Second, molecules are not mixed well in the spine. For instance, some receptors, enzymes, and scaffold proteins are elaborately arranged in the PSD to form a ‘signaling machine’ [103–105]. In particular, VGCCs, mGluRs, and PKC in the plasma membrane, and IP₃R in the ER, are tied together by PSD proteins [106, 107], which suggests that the entire machinery for coincidence detection of PF-CF inputs [49] is organized into a single huge protein complex. Calcium ions form a nanodomain or microdomain around a calcium channel, affecting only adjacent calcium effectors and localizing their signals [108, 109], whereas diffusion of the effec-

tors is important in information decoding of calcium spikes [110]. Finally, there are a variety of scaffolding proteins in the spine [105, 111, 112], and they might affect important properties of signaling cascades, such as sensitivity, specificity, and supralinearity [56, 63, 66, 113–115].

Some studies have addressed synaptic signaling and plasticity in conjunction with stochasticity, localization, and/or diffusion. CaMKII autophosphorylation is (controversially [116]) regarded as one of the candidates for long-term memory trace [117]. Its stability is likely to be limited by stochastic fluctuations, because there is an average of only 30 CaMKII holoenzymes per PSD [118]. Miller et al. [119] conducted Monte Carlo simulations [120] in order to elucidate factors that control switch stability, and to determine the functional relationship between stability and the number of molecules involved. The simulation demonstrated that the interplay between CaMKII and protein phosphatase I can form a bistable switch, whose stability depends exponentially on the number of enzyme molecules; the less the number of molecules, the more likely the switch is spontaneously turned on or off by stochastic fluctuations. The authors concluded that the number of CaMKII molecules found in the PSD (~ 30 [118]) is sufficient for the switch to potentially retain information for life.

Santamaria et al. [121] combined optical experiments and computer simulations in order to characterize molecular diffusion along PC dendrites. By locally photolysing a caged diffusion marker, fluorescein dextran (FD), and using confocal imaging [81], they visualized diffusion of FD within dendrites and demonstrated that diffusion was remarkably slower in spiny dendrites than in smooth dendrites. In spiny dendrites, the mean-square displacement of FD molecules did not increase linearly with time, but instead increased hyperbolically; in other words, diffusion appeared to gradually slow down. It was in contrast with normal diffusion observed in smooth dendrites. This retardation, known as ‘anomalous’ diffusion [122], in spiny dendrites may play an important role in neuronal computation by affecting the spatial and temporal distribution of signaling molecules. To understand its mechanisms, the authors modeled the realistic three-dimensional (3D) structure of PC dendrites with or without spines. Simulations revealed that dendritic spines act as traps for molecules, slowing down the diffusion process. While Santamaria et al.’s model successfully explained why diffusion was anomalous in spiny dendrites, more abstract and lower-dimensional models of a dendrite [89] (mentioned in a previous section) underesti-

mated the effects of spines on diffusion and failed to point at its anomalousness. This contrast may suggest the importance and advantage of realistic 3D simulation.

Neurotransmitters are released from the active zone of the presynaptic neuron, diffuse across the synaptic cleft, and bind to receptors at the opposing PSD. In some neurons, the transmitters are also released from sites outside the active zone and activate extrasynaptic receptors (ectopic release). To explore the significance of ectopic neurotransmission in the chick ciliary ganglion, Coggan et al. [123] simulated release of transmitter molecules as well as their diffusion and receptor binding by using a realistic 3D model. After exhaustive simulations, they concluded that most release was ectopic because otherwise the results would be inconsistent with experimental data.

Stochasticity, diffusion, and localization are very important factors in synaptic signaling and plasticity; however, the simulation of these factors is a huge computation load [124–126]. Nevertheless, Brownian motion of thousands of neurotransmitter molecules could be simulated exactly [123]. Other studies [127, 128] compromised between simulation speed and stochastic accuracy and utilized a method that dynamically chose between deterministic and stochastic calculations, depending on the number of molecules and propensity of forward reactions [129].

Conceptual Models of Long-Term Memory

The late phase of PF-PC LTD requires protein synthesis [4, 7, 21], but once consolidated, cerebellar memory is no longer protein synthesis-dependent [130]. Consolidated memories might be transferred from the cerebellar cortices to the deep cerebellar nuclei, as suggested by some animal studies on adaptation of vestibulo-ocular and optokinetic reflexes [131, 132]. But what is the final form of memory traces in the cerebellum whether they reside in the cortices or the nuclei?

1. Threshold Cascade Models

Retention of information requires stability of synapses, and new learning requires plasticity. Neurons need to solve the dilemma between stability and plasticity [50], but the question is ‘how?’ Fusi et al. [133] suggested a conceptual cascade model, in which synapses are binary and have two levels of strength: weak and strong, both of

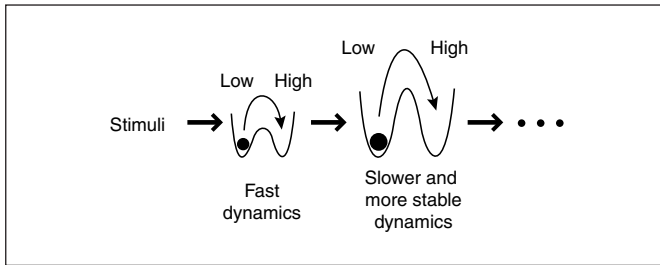


Fig. 4. Threshold cascade model. See text for explanation.

which are associated with their own cascade of multiple states. Whenever the conditions to reinforce the strength (i.e., applying an LTP induction protocol to a strong synapse and an LTD induction protocol to a weak synapse) are met, the state progresses further along the cascade, one step with each certain probability. Whenever the conditions to reverse synaptic strength (i.e., applying the LTD induction protocol to a strong synapse and the LTP induction protocol to a weak synapse) are met, the state jumps to the first step of the other cascade with another probability. The probability of transition depends on the depth of the current state within its cascade. The deeper the state is in the cascade, the more stable it is, and a transition is less likely to be made. The authors performed numerical simulations and demonstrated that (1) the plasticity and stability level that animals and humans possess is only possible with synapses whose states are linked by metaplastic transitions, and (2) the cascade model outperforms other similar models.

The Fusi et al. model provides an elegant theoretical solution for the stability versus plasticity dilemma; however, it unfortunately lacks a solid biological background. Taking into account the fact that bistability is ubiquitous in cellular systems [53, 54], Kawato [134] proposed a cascade of excitable and bistable dynamics (fig. 4). In his model, the fastest bistable system is activated by repetitive stimuli. The activated system then stimulates slower bistable dynamics that are more stable and require larger inputs for activation. In this way, activity is transmitted from one system to another slower one. Such a cascade of multiple bistable systems will form long-term memory that is stable, still open to modifications, and robust to noise.

This model is applicable to at least the first two steps of cerebellar LTD, i.e., Ca^{2+} -induced Ca^{2+} release and the MAPK-PKC positive feedback loop (fig. 1b). Ca^{2+} -induced Ca^{2+} release is a positive feedback loop, which is

activated by conjunctive PF-CF stimuli, resulting in a supralinear Ca^{2+} release [49] (fig. 1b-ii). The ON state lasts only 1 s, because IP_3Rs are inactivated by a high concentration of Ca^{2+} . The MAPK-PKC positive feedback loop is activated by repetitive inputs from the previous feedback loop, Ca^{2+} -induced Ca^{2+} release, and remains in the ON state for about 1 h [48, 72, 80] (fig. 1b-i).

2. Bidirectional Long-Term Memory Model

It is widely believed that memory is maintained in spine structures for extended periods in the brain [reviewed in 101, 112, 135–138], which might not be the case at least in the cerebellum and hippocampus. Sdrulla and Linden [139] demonstrated a surprising dissociation between LTD and spine morphology; chemically or synaptically evoked LTD in cerebellar slices was not associated with shrinkage or loss of dendritic spines. Manipulation that evoked significant spine retraction was not associated with LTD, either. A very similar ‘double dissociation’ between LTD and spine morphology was recently reported in the hippocampus [140]. In addition, spine structures can change very rapidly in a protein synthesis-independent manner [141], while, in contrast, long-term memory requires protein synthesis [4, 7, 21]. These findings suggest that long-term memory is retained by a mechanism other than spine morphology, and PKM ζ is probably the most likely candidate.

PKC isoforms are classified into three groups that differ in cofactor requirements: cPKCs, novel PKCs (nPKCs), and atypical PKCs (aPKCs). cPKCs require Ca^{2+} and DAG for activation; nPKCs are Ca^{2+} -independent and are activated by DAG alone; aPKCs are Ca^{2+} - and DAG-independent [reviewed in 142–144]. PKM ζ is a persistently active enzyme, consisting of the catalytic domain of an aPKC isoform, PKC ζ . It is expressed in a brain-specific manner in various regions, including the hippocampus and cerebellar cortex [145, 146]. A series of experiments performed mainly by Sacktor’s group demonstrates accumulating evidence that PKM ζ plays a pivotal role in long-term maintenance of memory. A procedure to induce hippocampal LTP first triggers transient activation of several PKC isoforms and translation of PKM ζ ; subsequently, PKM ζ protein increases and replaces PKCs in 30 min, and retains its activity for weeks [145, 147–150]. On the other hand, a procedure to induce hippocampal LTD reduces PKM ζ expression [151]. Injection of a PKM ζ inhibitor, ZIP [152], to the hippocampus reverses LTP maintenance in vivo and produces persistent loss of 1-day-old

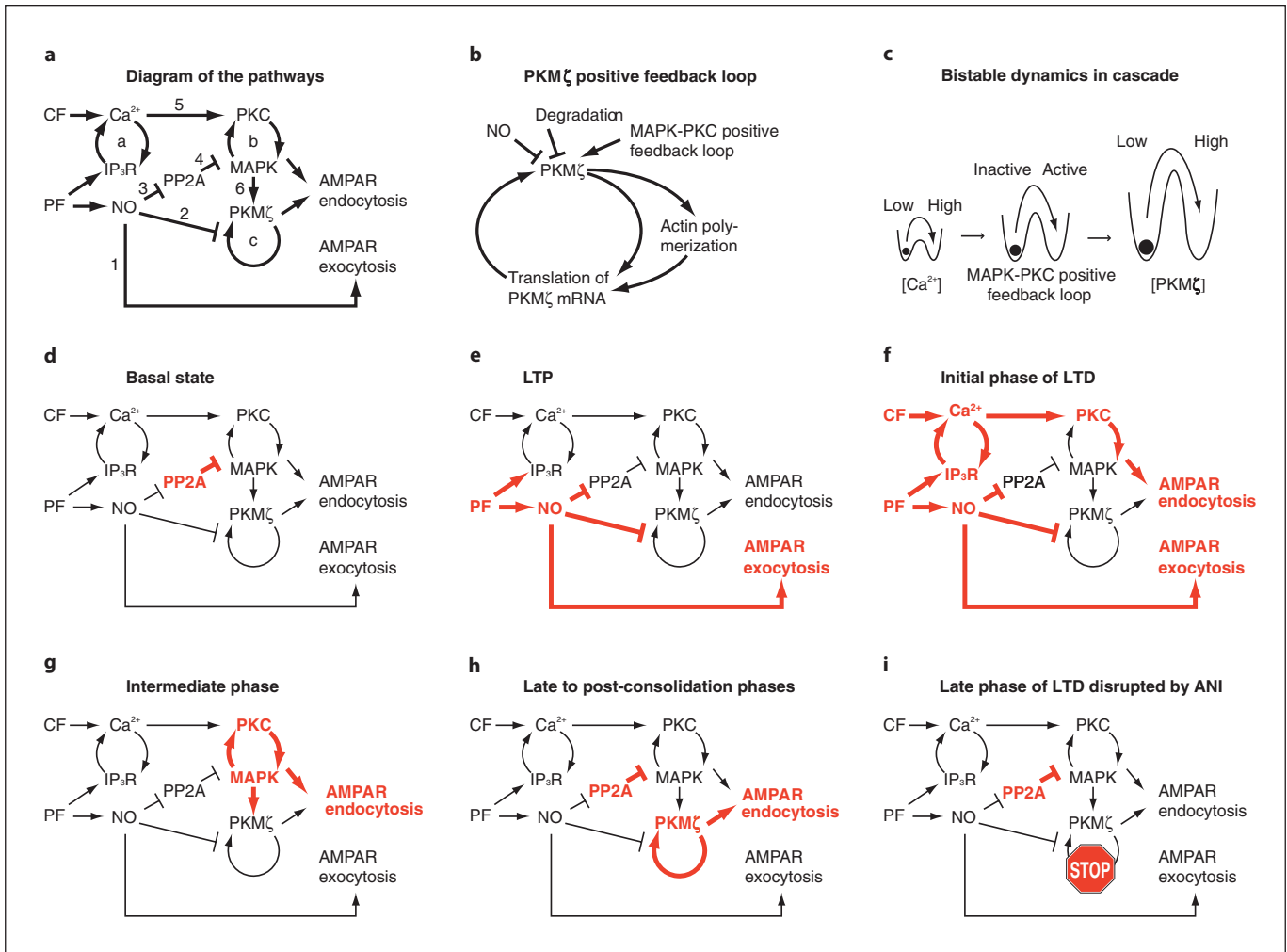


Fig. 5. Bidirectional long-term memory model. See text for explanation. **a** A schematic diagram of long-term memory. Sharp and blunt arrows indicate excitatory and inhibitory pathways, respectively. **b** The PKM ζ positive feedback loop. **c** The cascade model [134] representation of the bidirectional long-term memory model. **d-i** Various states of memory (red bold lines and black thin

lines indicate active and inactive pathways, respectively; red bold text and black thin text indicate active or increased molecules and inactive or decreased molecules, respectively): **d** the basal state, **e** LTP, **f** the initial, **g** intermediate, and **h** late to post-consolidation phases of LTD, and **i** the late phase of LTD disrupted by application of a protein synthesis inhibitor, such as anisomycin (ANI).

spatial information [153]. More surprisingly, an injection of ZIP to the rat neocortex, which is regarded as the long-term repository of memory, erases associative memories as old as 1 month [154]. During hippocampal LTP, various kinases, such as CaMKII, MAPK, PKC, and preexisting PKM ζ , regulate new synthesis of PKM ζ [155]; PKM ζ is likely to be locally translated in synaptodendritic domains [156–161]. These findings strongly suggest that PKM ζ is the (semi-)permanent form of a memory trace. Moreover, its importance to long-term memory is evolutionarily conserved from flies to rodents [149, 162]. PKM ζ

is also expressed in the cerebellar cortex [145, 146]. Since cerebellar plasticity is regarded as the mirror image of hippocampal plasticity, and shares much of its signaling cascade [4, 7, 23, 25, 163], PKM ζ activity is likely to be central to cerebellar LTD, as it is to hippocampal LTP.

We propose a conceptual model that explains the stability and plasticity of cerebellar memory, postulating that PKM ζ activity is the persistent memory trace (fig. 5). The model will also provide insight into hippocampal plasticity, which has much in common with cerebellar plasticity; however, it should be noted that [Ca²⁺] eleva-

tion and protein kinase activity induce opposite effects in the cerebellum and hippocampus; high $[Ca^{2+}]$ and active protein kinases result in LTD in the cerebellum and LTP in the hippocampus, while low $[Ca^{2+}]$ and inactive kinases result in LTP in the cerebellum and LTD in the hippocampus [25].

a. Pathways

PF firing induces NO synthesis. NO transiently enhances exocytosis of GluR2 through S-nitrosylation of *N*-ethylmaleimide-sensitive factor [164–167] (fig. 5a arrow 1). NO inactivates PKM ζ at the same time (fig. 5a arrow 2). This pathway is hypothetical, but there is a good reason to assume its existence; LTD is reversed by [NO] elevation which little $[Ca^{2+}]$ increase accompanies [27, 28, 164]. Although NO enhances AMPAR exocytosis, that would not be enough for LTD reversal if the activity to maintain LTD, PKM ζ activity in this model, were not turned off at the same time. PKM ζ inactivation might be mediated through nitration of PKM ζ or its activator, phosphoinositide-dependent protein kinase-1 [155].

NO also inhibits PP2A in an sGC-dependent manner (fig. 5a arrow 3) and reverses PP2A inhibition (fig. 5a arrow 4), allowing for activation of the MAPK-PKC positive feedback loop [4, 23, 168, 169]. Conjunctive firing of the PF and CF stimulates IP $_3$ Rs, resulting in Ca^{2+} -induced Ca^{2+} release (fig. 5a loop a). Ca^{2+} activates cPKC and PLA2 (fig. 1b, 5a arrow 5), triggering activation of the MAPK-PKC positive feedback loop (fig. 5a loop b) [4, 23, 72, 80, 142, 144]. The active forms of MAPK and PKC induce expression of PKM ζ [155] (fig. 5a arrow 6).

We postulate a positive feedback loop that activates PKM ζ translation in a PKM ζ -dependent manner (fig. 5a loop c, 5b) because PKM ζ activity is maintained for a surprisingly long period, much longer than the protein turnover timescale [147, 155]. The feedback loop consists of PKM ζ , actin polymerization, and local synthesis of PKM ζ , of which the latter two are PKM ζ -dependent. Since a single effector, PKM ζ , acts on two steps, i.e., actin polymerization and translation of itself, this results in ‘multistep ultrasensitivity’ [57]. As Ferrell [53] mentioned, the combination of supralinearity and a positive feedback loop can produce bistability and all-or-none responses. This part of the model is based on the following experiments: (1) PKM ζ mRNA is present in spiny dendrites [161]; (2) expression of PKM ζ during hippocampal LTP induction is very rapid, and this kinase is likely to be synthesized locally on demand [145, 147, 148]; (3) PKC activation results in actin polymerization in the neuron [170], and PKC ζ facilitates actin polymerization in various

types of cells [171–174]; (4) F-actin levels linearly regulate protein synthesis capacity of living cells [175], and (5) PKC and MAPK induce expression of PKM ζ [147, 148, 161].

In the active state of the PKM ζ positive feedback loop, endocytosis of AMPARs exceeds exocytosis, resulting in a depressed PF-PC synapse, whereas the inactive state of the feedback loop corresponds to a non-depressed synapse. As mentioned earlier, it should be noted that PKC activity (and presumably PKM activity as well) lead to the opposite effects in the cerebellum and hippocampus. In the PC, the Ser880 phosphorylation of GluR2 subunits by PKC results in endocytosis of AMPARs and reduction of transmission efficacy [4, 176, 177], while in the hippocampus, Ser818 phosphorylation of GluR1 subunits by PKC promotes synaptic incorporation of GluR1 and potentiation of synaptic transmission [178].

Central to the model are three positive feedback loops, i.e., Ca^{2+} -induced Ca^{2+} release, the MAPK-PKC positive feedback loop, and PKM ζ -induced PKM ζ expression. Their decay time constants are <1 s, tens of minutes, and weeks or longer, respectively. Importantly, these bistable dynamics of different time scales are connected in a cascade. When stimuli activate the quickest dynamics (fig. 5a loop a) repetitively, activity is transmitted from the quickest, to the intermediate (fig. 5a loop b), and thereafter to the slowest and most stable dynamics (fig. 5a loop c). As a consequence, long-term stability and plasticity of memory is established. In this sense, this model is a more concrete representation of the cascade model of excitable dynamics (fig. 4, 5c) [134].

b. Various States of Memory in the Model and Corresponding Experiments

In the basal state, PP2A is active and inhibits the MAPK-PKC positive feedback loop (fig. 5d).

i. LTP Induction

Postsynaptic LTP is induced in an NO-dependent manner by PF stimulation at 1 Hz, which increases [NO], but $[Ca^{2+}]$ only slightly [27, 33]. When a calcium chelate was infused to the PC, LTD-inducing stimuli resulted in LTP instead [29]. More surprisingly, LTP was induced in cerebellar slices just by applying an NO donor alone [164]. The model corresponds well to these experimental findings (fig. 5e). In the model, NO, whose synthesis is triggered by PF spikes, transiently facilitates exocytosis of AMPARs. Meanwhile, NO liberates the MAPK-PKC positive feedback loop from PP2A inhibition, but the loop is not activated because of the lack of Ca^{2+} inputs. PKM ζ ,

if present, is inactivated by NO. As a consequence, the synapse is potentiated.

By the way, NO donors, such as nitroglycerin, are prescribed for the treatment of ischemic heart disease [179, 180]. However, these drugs do not usually cause ataxia or amnesia by disturbing synapses. This may appear to contradict our model, but it can be explained by the fact that systemic administration of an NO donor does not increase [NO] in neurons sufficiently to activate their intracellular signaling cascades, even at a dose that considerably affects hemodynamics [181].

ii. Induction and Maintenance of LTD

The model explains the sequence of events during LTD induction and maintenance (fig. 5f–h). When $[Ca^{2+}]$ and [NO] are increased by conjunctive PF-CF activity, NO releases the MAPK-PKC positive feedback loop from PP2A inhibition, and Ca^{2+} activates cPKC, which then phosphorylates and internalizes AMPARs (fig. 5f). In the intermediate phase (fig. 5g), the activated MAPK-PKC positive feedback loop maintains PKC activity and endocytosis. During, and subsequent to, the late phase (fig. 5h), newly synthesized PKM ζ maintains AMPAR phosphorylation and endocytosis for a long period of time. This time course corresponds to Tanaka et al.'s experimental findings [72]. They showed that cerebellar LTD was reduced or abolished by a cPKC inhibitor, bisindolylmaleimide I (BIM), when applied within 30 min of induction. In contrast, BIM application at a later time point did not affect the LTD time course, which suggests that cPKC is vital for LTD in the initial and intermediate phases, but not in the late phase. Late-phase LTD maintenance must be BIM-insensitive, and PKM ζ is one of such PKC isoforms [182].

iii. Disruption of Late Phase LTD by Application of a Protein Synthesis Inhibitor

The model predicts that a protein synthesis inhibitor, such as anisomycin, disrupts late phase LTD by preventing expression of PKM ζ (fig. 5i), whereas it does not affect the initial and intermediate phases. Once PKM ζ is sufficiently expressed (post-consolidation phase), only minimal synthesis of PKM ζ is required in order to compensate for its degradation and maintain LTD. During this period, transient application of a protein synthesis inhibitor will fail to switch off the PKM ζ positive feedback loop, as long as the level of remaining PKM ζ is above the threshold for maintaining loop activity. These predictions correspond to *in vitro* and *in vivo* experiments. LTD induced in the presence of a protein synthesis in-

hibitor is transient and vanishes within an hour [183]. In eyeblink conditioning, a protein synthesis inhibitor prevents ongoing consolidation of new memory, but does not affect consolidated memory [130].

iv. Reactivation of Memory

It has been demonstrated in the cerebellum [130], as well as in the hippocampus and amygdala [184–186], that consolidated memory of conditioning training becomes labile following its retrieval. In order for reactivated memory to be maintained, it must be reconsolidated through a protein synthesis-dependent pathway. The model can also explain this retrieval-induced deconsolidation of memory. Reactivation sessions, which are very similar to the training sessions, will result in increased [NO] and $[Ca^{2+}]$ in the synapses that receive task-related inputs and store the conditioning memory (fig. 5f). NO inactivates the memory trace, PKM ζ , but at the same time the MAPK-PKC positive feedback loop is activated by Ca^{2+} and NO (fig. 5f, g). The typical steps of LTD induction are repeated, and this leads to new PKM ζ synthesis (fig. 5f→g→h). As a result, the memory is maintained, despite NO inactivation of preexisting PKM ζ . However, when a protein synthesis inhibitor is administered during retrieval sessions, the drug will block translation of PKM ζ (fig. 5i). Inactivation of preexisting PKM ζ and inhibition of new PKM ζ synthesis will shut down the PKM ζ loop activity and result in memory disruption. In contrast, co-administration of a protein synthesis inhibitor and a NOS inhibitor will not destroy memory, as predicted earlier [30]. It is because [NO] is kept at such a low level that existing PKM ζ will not be inactivated during the retrieval sessions.

c. Experiments to Verify the Model

First of all, it is necessary to determine whether PKM ζ maintains long-term memory in the cerebellum. If so, it should also be examined whether PKM ζ expression persists in the cerebellar cortex or shifts to the deep cerebellar nucleus, as experimentally suggested [187]. Second, according to the model, ZIP, a selective inhibitor of PKC ζ and PKM ζ [152], will abolish late phase LTD and associative memory, whereas overexpression of PKM ζ will result in LTD. The model predicts that cerebellar postsynaptic LTP is protein synthesis-independent, in contrast to LTD [4, 21, 23], since LTP corresponds to PKM ζ downregulation (fig. 5e). This could probably be tested *in vivo* as well as *in vitro*. In mice, cerebellar LTD and LTP have been shown to be cellular correlates of an increase and decrease in vestibulo-ocular reflex (VOR) gain, respective-

ly [188]. If the model is correct, a protein synthesis inhibitor, as well as ZIP, will abolish the increase in VOR gain, without affecting its decrease.

Future Perspectives

Recent advances in experimental strategies and techniques, such as conditional transgenic and gene-targeting technologies and proteomic profiling [104, 189–193], have revealed a myriad of molecules that participate in memory formation. It is now thought that the majority of essential mediators of synaptic plasticity have been identified, so the next topic of interest will be to understand how these molecules can form memory elements in the microenvironment of the dendritic spine, where the ef-

fects of stochastic noise, uneven localization, diffusion, and scaffolding are overwhelming. Efforts should also be made to untangle the intricate molecular interactions in the signaling networks [47, 55, 193–202]. To further our understanding of the fundamental mechanisms for synaptic plasticity, a combination of theoretical and experimental studies is very important.

Acknowledgements

We thank Prof. Erik De Schutter (Okinawa Institute of Science and Technology or OIST) for his valuable suggestions and advice, Mr. Stefan Wils (OIST) for fruitful discussion, and Ms. Miho Onizuka (Nara Institute of Science and Technology) for helpful comments on the manuscript. This work was supported by a grant from the Uehara Memorial Foundation.

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