Systems Biology Perspectives on Cerebellar Long-Term Depression

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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 (AMPARs) 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 CF inputs, forming the molecular basis for associative learning; 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. Neurons 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 pair of positive feedback
loops, a quick one of MAPK and PKC, and a slower one of protein kinase Mζ (PKMζ)-activated PKMζ synthesis, generates stable memory for a very long period of time that is still amenable to modifications.

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 organism for supervised learning (also known as associative learning, in 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 input: one from hundreds of thousands of PFs and the other from a single CF. Marr-Albus-Ito theory [10-12] states that the neuronal circuit they make underlies associative learning. In the theory, PFs provide a sensorimotor context to the PCs, while CFs carry either teaching or error signals that modify PF-PC synapses in a supervised 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 studies have revealed that LTD is regulated by [Ca²⁺] [14-16] and that [Ca²⁺] elevation and PF-PC LTD
are most prominent when PF activity precedes CF activity by 50 – 250 ms [17, 18] (N.B.: In this article, [substance] stands for the concentration of the substance). The optimal time window for \([\text{Ca}^{2+}]\) elevation and LTD induction \textit{in vitro} (50 – 250 ms) is comparable with the \textit{in vivo} CF input delay with respect to PF inputs (~100 ms) [3-5, 11] that is produced by the definite sequence of events taking place during motor execution (more specifically, first, PCs receive PF inputs, motor commands are released 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 \textit{in vitro} time window size and \textit{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 \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors (AMPARs), which takes place when activated protein kinase C (PKC) phosphorylates GluR2 subunits of the receptors. The molecules and the signaling networks participating in cerebellar LTD have been extensively reviewed [4, 7, 21-26]. Essential molecules include mitogen-activated protein kinase (MAPK), MAPK kinase (MAPKK), MAPKK kinase (MAPKKK), PKC, phospholipases A2 (PLA2) and C (PLC),
arachidonic acid (AA), inositol 1,4,5-triphosphate (IP$_3$), and many others.

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. Presynaptically synthesized diffusible messenger nitric oxide (NO) is a crucial “gatekeeper” [30] for cerebellar plasticity: LTD and LTP are induced by Ca$^{2+}$ and other signaling molecules 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 in the cerebellum [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], 82 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 previously claimed [47], in order to better characterize the molecular mechanisms for PF-PC plasticity and its computational role, it is very important to distinguish the minimal set of mediators that are required for the process of memory formation from
modulators; these modulators alter the process, yet are nonessential.

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 eventually 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$_3$R) 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 the systems biology of cerebellar LTD, mainly focusing on our own published work, followed by a discussion of the possible effects of stochasticity, localization, diffusion, and scaffolding on the signaling cascade of synaptic signaling 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 this stability versus plasticity dilemma. In the final section, we propose a conceptual model in which the MAPK- PKC positive feedback loop induces cerebellar LTD, while another positive feedback loop of protein kinase M$_{\zeta}$ (PKM$_{\zeta}$)-activated PKM$_{\zeta}$ synthesis
maintains long-term memory. The model predicts the network to be stable for a very long period of time, but still amenable to modifications.

Simulation studies on cerebellar LTD

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 the Michaelis-Menten kinetics to changes in the stimuli amplitude; it 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 1950’s, autocatalytic enzymes were predicted to have multistable steady states [58]. Conceptual models were proposed in the 1980’s 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 biology has revealed that the MAPK cascade is very important for 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, untrasensitivity arises also when two opposing enzymes (e.g., a kinase and a phosphatase) operate at near-saturation levels, and the reaction rates are almost independent of the substrate level (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] (see also [71]).

Kuroda et al. [48] hypothesized that the MAPK cascade and other enzymes in the PC, eg., PLA2 and PKC, make a positive feedback loop, which ultimately 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 \([Ca^{2+}]\) and [diacylglycerol (DAG)] in dendritic spines of the PC. \(Ca^{2+}\) and DAG activate conventional PKC (cPKC), a PKC
isoform that is sensitive to both Ca$^{2+}$ and DAG. Activated cPKC phosphorylates AMPARs, and phosphorylated receptors are eventually removed from the postsynaptic membrane through endocytosis. Thus, the magnitude of LTD corresponds to AMPAR phosphorylation. cPKC also activates Raf, a MAPKKK, and Raf activates MEK, a MAPKK, through dual phosphorylation. And finally, MEK activates MAPK also through dual phosphorylation. Either Ca$^{2+}$ or activated MAPK 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). 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 linear cascades of 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 minutes. There was a sharp threshold level of input stimuli, and weak stimuli did not result in AMPAR phosphorylation; however, stimuli that were stronger than the threshold stereotypically resulted in phosphorylation of a large amount of receptors. 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]. According to previous studies [68, 69], the ultrasensitivity of the MAPK cascade is very important for the all-or-none property of the feedback loop, and the Kuroda et al. model is in accordance with this. Fig. 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. [active PP2A] was kept constant at 85 nM, and total concentrations of Raf, MEK, and MAPK were 0.5 μM, 0.5 μM, and 1 μM, respectively). 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).

AMPAR phosphorylation in the Kuroda et al. model is transient 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 (data was produced for this review article; [active PP2A] was kept constant at 85 nM). Phase plane profiles are curves of one dependent variable against another and are used to determine whether the 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 fig. 2b: one is [active cPKC] against [MAPK-PP] (solid bold line), and the other is [MAPK-PP] against [active cPKC] (dotted 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 indicates that the supralinear response of [MAPK-PP] against [active PKC] (dotted line) 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’s.

Experimental verification followed the theoretical study. A series of slice experiments performed by Tanaka et al. [72, 80] revealed that the MAPK-PKC positive feedback loop is central to PF-PC LTD and responsible for its supralinearity. First, they demonstrated that MAPK acts downstream of PKC. MAPK was activated by an LTD induction protocol in the absence of a PKC inhibitor, while in its presence, MAPK was not activated by the protocol: in the presence of a MAPK inhibitor, a PKC activator failed to induce LTD. 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 photolysing caged calcium and using confocal imaging [81] to visualize ramp-like rises in $[\text{Ca}^{2+}]$, it was subsequently demonstrated that elevation of $[\text{Ca}^{2+}]$ alone is sufficient for the induction of cerebellar LTD; the
relationship between [Ca\textsuperscript{2+}] and LTD was quantified for the first time. LTD was induced stereotypically by [Ca\textsuperscript{2+}] elevations of substantial peak and duration, while small or short-term elevations in [Ca\textsuperscript{2+}] resulted in almost no LTD (fig. 3a). The sigmoidal relationship between peak [Ca\textsuperscript{2+}] and the amount of LTD (each curve in fig. 3a) was so supralinear that it could be described by the Hill equation using a large Hill coefficient value of five (fig. 3a). Even though LTD was a supralinear function of [Ca\textsuperscript{2+}], the all-or-none property, which Kuroda et al. [48] had predicted, was not observed. The discrepancy between the experiments and simulations is probably due to spatial gradients in [Ca\textsuperscript{2+}] that occurred during local uncaging, the errors in experimental measurements, and stochastic fluctuations in spines. It is reminiscent of previous experiments demonstrating that individual synaptic plasticity is discrete and heterogeneous, while these synapses present graded plasticity as a whole [75]. To compare the experiments with model predictions, the Kuroda et al. model was modified for a special form of LTD that is induced solely by Ca\textsuperscript{2+}, without increases in DAG or NO. By further refining the model so that it took into consideration spatial heterogeneity of stimuli, errors, and noise, the model accurately simulated experimental measurements (fig. 3b). These results indicate how useful this model can be to predict and explain future experiments.

In order to verify that the MAPK-PKC positive feedback loop (fig. 1b) is the
underlying mechanism for supralinearity of LTD, the authors examined the relationship between $[\text{Ca}^{2+}]$ 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 (qualitative and quantitative) 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 $[\text{Ca}^{2+}]$ for LTD induction was a time-dependent variable. The longer the duration of a $\text{Ca}^{2+}$ stimulus was, the lower the necessary $[\text{Ca}^{2+}]$ 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 $[\text{Ca}^{2+}]$, the integrated amount of required $[\text{Ca}^{2+}]$ for LTD remained time-dependent and increased over time (fig. 3e,f), which suggests that the time integration process in LTD is somewhat leaky. Such a leaky integration of $[\text{Ca}^{2+}]$ can be described mathematically as
\[
\tau \frac{dx}{dt} = -x + a[Ca^{2+}](t)
\]  
(1)

where \( a \) is a scaling factor, \( \tau \) 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 \([Ca^{2+}]\) is elevated in a ramp-like fashion as in [80], the solution of Equation 1 is

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

where \( k \) is the rate of increasing \([Ca^{2+}]\) (peak \([Ca^{2+}]\) divided by the uncaging duration, \( t \)). After these parameters were paired to the experimental results (\( a \) and \( \tau \) were 18.7%/\( \mu \)M and 0.56 sec, respectively), the amount of LTD was plotted against \( x \) in fig. 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 fig. 3a,b,e,f verifies Equation 2 and supports the hypothesis that the signaling processes of LTD behave as a leaky integrator of \([Ca^{2+}]\).

**Coincidence detection of cerebellar inputs**

PF-PC plasticity is thought to be the cellular process of cerebellar associative learning [10-12], but what molecular mechanisms enable the PF-PC synapse to sense coincident activities of PF and CF within the time frame? 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\textsuperscript{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 [Ca\textsuperscript{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. Scenarios a-c cannot explain the ~100 ms' PF-CF delay that is optimal for [Ca\textsuperscript{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.

How suitable is the IP\textsubscript{3}R (e) for coincidence detection, then? IP\textsubscript{3}Rs are located in a calcium store of the dendritic spine, the endoplasmic reticulum (ER). The receptor is synergistically co-activated by Ca\textsuperscript{2+} and IP\textsubscript{3}, and releases a large amount of Ca\textsuperscript{2+} from the ER in response. The open probability of IP\textsubscript{3}R displays a bell-shaped curve for dependence on calcium because a large [Ca\textsuperscript{2+}] is inhibitory to the receptor [84, 85]. As shown in fig 1b-ii, on the one hand, PF firing activates PLC through the mGluR1 metabotropic glutamate receptor pathway, resulting in production of IP\textsubscript{3} (and DAG) from phosphatidylinositol bisphosphate. On the other hand, CF firing depolarizes the PC and induces influx of Ca\textsuperscript{2+} through VGCCs. Therefore, [IP\textsubscript{3}] and [Ca\textsuperscript{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], IP$_3$R may act as a coincidence detector that associates PF inputs (desired trajectories and sensory feedback) with CF inputs (error signals [3]). However, massive stimulation of a PF bundle alone [86, 87], or uncaging either Ca$^{2+}$ or IP$_3$ [16, 88], can also induce LTD, which raises doubts that LTD is one of the cellular bases 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 fig. 1b·ii, the model consists mainly of mGluR1s, Gq protein, PLC in the postsynaptic density (PSD), IP$_3$Rs 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 previously published work. Their simulation demonstrated that the supralinear Ca$^{2+}$ response to conjunctive PF-CF inputs is a regenerative process that is driven by IP$_3$R. In other words, [Ca$^{2+}$] elevation opens IP$_3$Rs, and outflow of Ca$^{2+}$ from the ER results in additional [Ca$^{2+}$] elevation (Ca$^{2+} \rightarrow$ IP$_3$Rs$\rightarrow$Ca$^{2+}$), thereby producing a positive feedback loop. In addition, PLC is not responsible for the supralinearity of Ca$^{2+}$ influx. In theory, [Ca$^{2+}$] elevation may enhance the activity of PLC for IP$_3$ production, and IP$_3$ elevation may open IP$_3$Rs to release Ca$^{2+}$ from the ER, creating another positive feedback
loop (Ca$^{2+} \rightarrow$ PLC $\rightarrow$ IP$_3$ $\rightarrow$ IP$_3$Rs $\rightarrow$ Ca$^{2+}$). However, simulations have demonstrated that this hypothetical pathway does not form a working positive feedback loop. 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 [Ca$^{2+}$] for Ca$^{2+}$-induced Ca$^{2+}$ release, and the threshold is regulated by [IP$_3$]. In line with previous experiments [17], simulations demonstrated that a CF input within 300 ms after PF inputs induced a Ca$^{2+}$ transient, which easily reached the threshold of [Ca$^{2+}$] for regenerative Ca$^{2+}$ release, because the threshold level was already lowered by IP$_3$. In contrast, when a CF input occurred either before PF inputs or too late after PF inputs, the CF-mediated elevation of [Ca$^{2+}$] failed to reach the threshold, because the threshold had already returned to a higher level once [IP$_3$] decreased. The IP$_3$-dependent Ca$^{2+}$ threshold is capable of explaining not only conjunctive LTD, but also LTD in non-physiological conditions. Even at the baseline [IP$_3$], where the Ca$^{2+}$ threshold for Ca$^{2+}$-induced Ca$^{2+}$ release is highest, an excessive increase in [Ca$^{2+}$] could reach the threshold and induce LTD, as seen with massive activation of a PF bundle [86, 87] and Ca$^{2+}$ uncaging [16]. Similarly, an
extremely high [IP$_3$], as seen upon IP$_3$ uncaging [88], would result in LTD without requiring an increase in [Ca$^{2+}$], because the baseline [Ca$^{2+}$] is already greater than the IP$_3$-regulated Ca$^{2+}$ threshold. The extensive descriptive ability of the Ca$^{2+}$ kinetics model indicates that the model captures the essence of the molecular mechanisms of cerebellar LTD.

Hernjak et al. [89] simulated the Ca$^{2+}$ kinetics of PC dendrites and spines with realistic parameters, similarly to Doi et al. This study was unique, because they considered one- or two-dimensional diffusion and localization of molecules in addition to biochemical processes. They demonstrated that high density and low sensitivity of IP$_3$Rs, which are characteristics of PCs [90], are critical for generating and localizing Ca$^{2+}$ spikes in a single spine. Ca$^{2+}$ was compartmentalized in the spine by the narrow spine neck, as shown in previous studies [91-93], while IP$_3$ freely diffused into the dendrite and neighboring spines.

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, and that 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 PC signaling pathways (fig. 1b-i) [48, 49, 97], and further characterized the role of NO in cerebellar learning. The simulation results revealed that NO, whose concentration was dependent on surrounding PF activity, regulated LTD. During low PF activity in the vicinity, conjunctively stimulated PF–PC synapses were incapable of undergoing LTD, due to insufficient NO concentration. During excessive PF activity, even neighboring synapses underwent LTD, due to excessive amounts of NO, which resulted in loss of synaptic specificity. LTD occurred in a synapse-specific manner only with moderate levels of nearby PF activity. Based on these results, the authors proposed a hypothesis that NO enables context-dependent selection of appropriate learning modules. Another prediction was that any movement made in any context was encoded by a small percentage of PFs, otherwise LTD did not occur at all or was unspecific. An animal experiment was suggested to verify their hypothesis. 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 simple cells, whose cytoplasm and plasma membrane are assumed to be relatively homogeneous, such as *Xenopus* oocytes and undifferentiated culture cells [67, 68, 98]; however, it may not always be appropriate in neurons for several reasons. First, the spine of an excitatory synapse 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 few as 4-73 [102]. In such cases, stochastic fluctuations come into play, and mass-action kinetics, described in continuous equations, are no longer applicable. Second, molecules are not mixed well in the spine. For instance, receptors, enzymes, and scaffold proteins are organized in the PSD and form “signaling machines” [103-105]. In particular, VGCCs, mGluRs, and PKC in the plasma membrane, and IP₃Rs in the ER, are tied together by PSD proteins [106, 107], which suggests that the entire pathway for coincidence detection of PF-CF inputs [49] is organized in 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], while diffusion of the effectors is important in information decoding of calcium spikes [110]. Finally, there are a myriad of scaffolding proteins in the spine [105, 111, 112], and they might theoretically affect important properties of signaling cascades, such as sensitivity, specificity,
and supralinearity [56, 63, 66, 113-115]. Several 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]. Stability is likely to be limited by stochastic fluctuations, because there are 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 a very stable switch to potentially retain information for life.

Santamaria et al. [121] combined optical experiments and computer simulations 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 in spiny dendrites was remarkably slower than that in smooth dendrites. In spiny dendrites, the mean-square displacement of FD molecules did not increase linearly with time, as expected for normal diffusion, but instead increased hyperbolically; in other words, the diffusion coefficient appeared to gradually slow down over time. In order to understand the mechanisms for this retardation, known as “anomalous” diffusion [122], the authors modeled the realistic three-dimensional structure of dendrites with or without spines. Simulations revealed that dendritic spines act as traps for molecules diffusing into them, slowing down the diffusion process in spiny dendrites. In contrast, diffusion was normal in smooth dendrites. Next, they returned to experiments and showed anomalous diffusion of a signaling molecule, IP$_3$, in spiny dendrites, which was expected from their previous simulations and experiments. Unlike FD diffusion, IP$_3$ diffusion was anomalous also in smooth dendrites to a lesser extent, probably due to intracellular binding and degradation. This study suggests that anomalous diffusion in PC dendrites has a computationally important role because it shapes intracellular signaling and interaction of molecules within spines. Santamaria et al.’s successful demonstration of anomalous diffusion in their simulations probably owes to their three-dimensional model because a two-dimensional PC dendrite model [89] (mentioned in a previous section) underestimated the effects of spines on diffusion and failed to point at its “anomalousness".
Coggan et al. [123] developed a realistic three-dimensional model of the chick ciliary ganglion in order to explore the significance of ectopic neurotransmission. They simulated release of neurotransmitters from presynaptic terminals and ectopic locations, as well as their diffusion and receptor binding. The results demonstrated that most transmitter release should be ectopic in order for results to be consistent 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 cerebellar LTD requires protein synthesis [4, 7, 21]; however consolidated memory that is older than several days is no longer protein synthesis-dependent [130]. Consolidated memories might be transferred from the cerebellar cortices to the deep cerebellar nuclei, as
shown in several animal studies on adaptation of vestibulo-ocular and optokinetic reflexes [131, 132]. What is the final form of memory traces in the cerebellum, regardless of whether they reside in the cortices or the nuclei?

Threshold cascade models

Retention of information requires stability, 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 which are associated with their own cascade of multiple states. Whenever the conditions to reinforce the strength are met (i.e., applying an LTP induction protocol to a strong synapse and an LTD induction protocol to a weak synapse), the state progresses further along the cascade, one step with each certain probability. Whenever the conditions to reverse synaptic strength are met (i.e., applying the LTD induction protocol to a strong synapse and the LTP induction protocol to a weak synapse), 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 it is less likely that a transition will be made. The authors demonstrated that 1) the plasticity and stability level that animals and humans possess is only possible with synapses that demonstrate a wide degree of plasticity 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. 1,4). 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] (figs. 1b-ii, 4). The ON state lasts only one second, because IP$_3$R is inactivated by a large amount of Ca$^{2+}$. The MAPK-PKC positive feedback loop is activated by repetitive inputs from the previous feedback loop, i.e., Ca$^{2+}$-induced Ca$^{2+}$ release, and remains in the ON state for about one hour.
[48, 72, 80] (fig. 1b-i, 4).

**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. 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 has recently been reported in the hippocampus [140]. In addition, spine structures can change very rapidly in a protein synthesis-independent manner [141]. These findings suggest that long-term memory is retained by a mechanism other than spine morphology, and PKMζ 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]. PKMζ is a persistently active enzyme, consisting of the catalytic domain of an aPKC isoform, PKCζ. It is expressed in a brain-specific
manner in various regions, including the hippocampus and the cerebellar cortex [145, 146], and is indispensable for long-term memory in mammals and Drosophila [147, 148]. A series of experiments performed mainly by Sacktor’s group demonstrates accumulating evidence that PKMζ plays a pivotal role in long-term maintenance of memory. A procedure to induce hippocampal LTP triggers, during the first 30 min, transient activation of several PKC isoforms and the translation of PKMζ; subsequently, PKMζ gradually increases and replaces PKCs, resulting in the maintenance of LTP for weeks [145, 147, 149-151]. On the other hand, a procedure to induce hippocampal LTD reduces PKMζ expression [152]. Injection of a PKMζ inhibitor, ZIP [153], to the hippocampus reverses LTP maintenance in vivo and produces persistent loss of 1-day-old spatial information [154]. 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 one month [155]. During hippocampal LTP, various kinases, such as CaMKII, MAPK, PKC, and preexisting PKMζ, regulate new synthesis of PKMζ [156]; PKMζ is likely to be locally translated in synaptodendritic domains [157-162]. These findings suggest that PKMζ is the (semi-)permanent form of a memory trace. PKMζ 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], it is likely that PKMζ plays a pivotal role in cerebellar LTD, as well as in hippocampal LTP.
We propose a conceptual model that explains the stability and plasticity of cerebellar memory, postulating that PKM\textsubscript{\textgreek{z}} activity is the persistent memory trace (fig. 5a). The model will also provide insight into hippocampal plasticity, which has much in common with cerebellar plasticity; however, it should be noted that [Ca\textsuperscript{2+}] elevation and protein kinase activity induce opposite effects in the cerebellum and hippocampus; in other words, high [Ca\textsuperscript{2+}] and active protein kinases result in LTD in the cerebellum and LTP in the hippocampus, while low [Ca\textsuperscript{2+}] and inactive kinases result in LTP in the cerebellum and LTD in the hippocampus [25].

Pathways
The central part of the model consists of two positive feedback loops, the MAPK-PKC positive feedback loop and another slower loop of PKM\textsubscript{\textgreek{z}}-activated PKM\textsubscript{\textgreek{z}} synthesis. NO transiently enhances exocytosis of GluR2 through S-nitrosylation of N-ethylmaleimide-sensitive factor [164-167] (fig. 5a arrow 1). NO inactivates PKM\textsubscript{\textgreek{z}} at the same time (fig. 5a blunt arrow 2). This hypothetical pathway is probably mediated through nitration of PKM\textsubscript{\textgreek{z}} or its activator, phosphoinositide-dependent protein kinase-1 [156]. NO also inhibits PP2A in an sGC-dependent manner (fig. 5a blunt arrow 3) and reverses PP2A inhibition (fig. 5a blunt arrow 4), allowing for activation of the MAPK-PKC positive feedback loop [4, 23, 168, 169]. Ca\textsuperscript{2+} activates cPKC
(fig. 5a arrow 5) and PLA2 (fig. 1b), triggering activation of the MAPK-PKC positive feedback loop in the presence of NO (fig. 5a circle 6) [4, 23, 72, 80, 142, 144]. The activated loop induces expression of PKMζ [156] (fig. 5a arrow 7).

We postulate a positive feedback loop that activates PKMζ translation in a PKMζ-dependent manner (fig. 5a circle 8, 5b), because PKMζ activity is maintained for a surprisingly long period, much longer than the protein turnover timescale [149, 156]. The feedback loop consists of PKMζ, actin polymerization, and local synthesis of PKMζ, of which the latter two are PKMζ-dependent. Since a single effector, PKMζ, acts on two steps, i.e., actin polymerization and translation of itself, this results in "multistep ultrasensitivity" [57]. As Ferrell [53] refers to, 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) PKMζ mRNA is present in the PC spiny dendrite [146]; 2) expression of PKMζ during hippocampal LTP induction is very rapid, and this kinase is likely to be synthesized locally on demand [145, 149, 150]; 3) PKC activation results in actin polymerization in the neuron [170], and PKCζ 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 PKMζ [149, 150, 162],
In the active state of the PKMζ positive feedback loop, endocytosis of AMPARs exceeds exocytosis, which results in a depressed PF-PC synapse, while the inactive state of the feedback loop corresponds to a non-depressed synapse. Although it is rather contradictory, PKC activity leads to the opposite effect 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].

The central part of this model is a pair of positive feedback loops, the MAPK-PKC loop with a time constant of several tens of minutes and the slower PKMζ loop with a time constant of days, weeks, months, or maybe even years. It is important to note that prior to the MAPK-PKC loop there is a faster positive feedback loop with a time constant of less than one second, which is Ca^{2+}-induced Ca^{2+} release mediated by IP₃Rs [49]. Importantly, three bistable positive feedback loops of different time scales are connected in a cascade. When stimuli activate the quickest dynamics repetitively, activity is transmitted from the quickest, to the intermediate, and thereafter to the slowest dynamic. 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 bistable dynamics (fig. 4) [134].

Various states of memory in the model and corresponding experiments

**LTP induction**

PF stimulation alone induces presynaptic synthesis of NO, which inactivates PKMζ and transiently facilitates exocytosis of AMPARs (fig. 5c). Meanwhile, NO liberates the MAPK-PKC positive feedback loop from PP2A inhibition; however, the loop is not activated because of the lack of Ca\(^{2+}\) inputs. As a consequence, the synapse is potentiated. This situation corresponds to previous experimental findings. PF stimulation at 1 Hz, which increases [NO], but [Ca\(^{2+}\)] only slightly, induces postsynaptic LTP in a NO-dependent manner [27, 33]. When a calcium chelate is infused to the PC, LTD-inducing stimuli result in LTP instead [29]. More surprisingly, a bath application of an NO donor alone induces LTP [164].

NO donors, such as nitroglycerin, are prescribed for many patients with ischemic heart disease [179, 180]: of course, these drugs do not usually cause ataxia or amnesia in these patients by disturbing synapses. This can probably 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].
**Induction and maintenance of LTD**

When \([\text{Ca}^{2+}]\) and [NO] are increased simultaneously, NO releases the MAPK-PKC positive feedback loop from PP2A inhibition, and \(\text{Ca}^{2+}\) activates cPKC, which then phosphorylates and internalizes AMPARs (fig. 5d). In the intermediate phase (fig. 5e), the activated MAPK-PKC positive feedback loop maintains PKC activity and endocytosis. During, and subsequent to, the late phase (fig. 5f), newly synthesized PKM\(\zeta\) maintains AMPAR phosphorylation and endocytosis for a long period of time. Tanaka et al. [72] reported 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 the maintenance of LTD in the initial and intermediate phases, but not in the late phase. Late-phase LTD maintenance must be BIM-insensitive, and PKM\(\zeta\) is one of these PKC isoforms [182].

**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\(\zeta\) (fig. 5g), whereas it does not affect the initial and intermediate phases. Once PKM\(\zeta\) is
sufficiently expressed (post-consolidation phase), further synthesis of PKMζ is only necessary in order to compensate for its degradation. During this period, transient application of a protein synthesis inhibitor fails to switch off the PKMζ-positive feedback loop, as long as the level of remaining PKMζ 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 inhibitor 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].

**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 memory retrieval sessions. In order for reactivated memory to be maintained, it must be reconsolidated through a protein synthesis-dependent pathway. The model can also explain retrieval-induced deconsolidation of memory. Reactivation sessions result in increased [NO] and inactivated PKMζ in synapses that store the conditioning memory (fig. 5d). At the same time, an increase in [Ca^{2+}] and [NO] activates the MAPK-PKC positive feedback loop (fig. 5d,e). Since the typical steps of LTD induction are repeated, this leads to new PKMζ synthesis (fig. 5d→e→f). As a result, the memory is maintained, despite NO inactivation of preexisting
PKMζ. However, when a protein synthesis inhibitor is administered during retrieval sessions, the drug will block translation of PKMζ (fig. 5g), leading to loss of PKMζ activity and memory disruption. This model is also in agreement with a previous prediction that co-administration of a protein synthesis inhibitor and a NOS inhibitor does not disrupt reactivated memories [30]. In this situation, [NO] is pharmacologically kept at a low level; therefore, retrieval sessions do not inactivate existing PKMζ nor result in labile memories.

Experiments to verify the model

It is necessary to determine whether PKMζ maintains long-term memory in the cerebellum through the use of an atypical PKC-selective inhibitor, ZIP [153], *in vitro* and *in vivo*. The model predicts that cerebellar postsynaptic LTP is protein synthesis-independent, in contrast to LTD [4, 21, 23], since LTP corresponds to PKMζ downregulation (fig. 5c). This could probably be tested by *in vivo* experiments. In mice, cerebellar LTD and LTP have been shown to be cellular correlates of an increase and decrease in vestibulo-ocular reflex (VOR) gain, respectively [187]. If the model is correct, administration of ZIP, or a protein synthesis inhibitor, during adaptation 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, as well as proteomic profiling [104, 188-192], have revealed a myriad of molecules that participate in synaptic plasticity. 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 form memory elements in the microenvironment of the dendritic spine, where the effects 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, 192-201]. To further our understanding of the fundamental mechanisms for synaptic plasticity, a combination of theoretical and experimental studies is very important.

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**Figure Legends**

**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 [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.

**Fig. 2 Suprilinearity 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. b) Phase plane analysis of the MAPK-PKC positive feedback loop. 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 dotted 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).

Fig. 3 Integration of $[\text{Ca}^{2+}]$ during LTD

Results of $a, c, e, g$) experiments and $b, d, f, h$) simulations are shown side by side. $a, b$) Relationship between peak $[\text{Ca}^{2+}]$ and LTD for different durations of $[\text{Ca}^{2+}]$ elevation. $c, d$) Relationship between peak $[\text{Ca}^{2+}]$ and LTD for different durations of $[\text{Ca}^{2+}]$ elevation in the presence of a PLA2 inhibitor. $e, f$) Relationship between integrated amount of $[\text{Ca}^{2+}]$ 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 [80] with permission from Elsevier Inc.

Fig. 4 Threshold cascade model

See text for explanation.

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. The blunt arrow 2 is dotted to indicate a hypothetical pathway. $b$) The PKM$\zeta$
positive feedback loop. c-g) Various states of memory. Red bold lines and black thin lines indicate activated and inactivated pathways, respectively; red bold text and black thin text indicate activated or increased molecules and inactivated or decreased molecules, respectively. c) LTP. d) The initial, e) intermediate, and f) late to post-consolidation phases of LTD. g) The late phase of LTD disrupted by application of a protein synthesis inhibitor, such as anisomycin (ANI).

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Fig. 1
Active PKC (µM) vs. Downstream molecules (µM)

Fig. 2
Experiments

Model

Experiments

Model

Experiments

Model

Experiments

Model

Experiments

Model

Stimuli → [Ca\textsuperscript{2+}] → MAPK-PKC positive feedback → ?

Fig. 4
Fig. 5