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Bistable Switches for Synaptic Plasticity

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A persistent decrease in synaptic efficacy, called long-term depression (LTD), of the parallel fiber–Purkinje cell synapse is thought to underlie some forms of learning and memory in the cerebellum. Simulation studies predicted that mitogen-activated protein kinase and protein kinase C would mutually activate each other and make a bistable positive feedback loop, thereby providing a molecular basis for LTD. This claim stimulated experimenters to successfully demonstrate the feedback loop and its pivotal role in cerebellar LTD.

What is the most fundamental process of learning and memory? An appealing early theory (*1*, *2*) stated that positive feedback loops of enzymatic reactions that are bistable and maintain activity despite molecular turnover may provide a basis for cellular memory. In 1999, Bhalla and Iyengar (*3*) computationally analyzed potential positive feedback loops present in the larger network of interacting real signaling pathways and showed that these loops could actually function as bistable switches and store information. This theoretical study inspired Tanaka and Augustine (*4*), who experimented on brain slices and demonstrated that a positive feedback loop of enzymes actually underlies synaptic plasticity of the cerebellum. This pair of studies, starting with Bhalla and Iyengar (*3*) and concluding with Tanaka and Augustine (*4*), exemplifies the potential of the combination of theoretical and experimental studies for deepening our insight into signaling networks.

Intracellular signaling pathways of neurons have been intensively studied. One such pathway involves mitogen-activated protein kinase (MAPK), which phosphorylates and activates phospholipase A_2 (PLA_2), which in turn produces the chemical mediator arachidonic acid (AA). AA activates protein kinase C (PKC), which activates the MAPK cascade, consisting of MAPK kinase kinase, MAPK kinase, and MAPK. This appears to constitute a positive feedback loop (the MAPK-PKC positive feedback loop; MAPK cascade → PLA_2 → AA → PKC → MAPK cascade) (Fig. 1A). Bhalla and Iyengar (*3*) computationally investigated emergent properties of this MAPK-PKC signaling network. Simulations with realistic parameter values confirmed that the MAPK cascade, PLA_2, and PKC actually interacted to form a positive feedback loop, which responded to stimuli in an all-or-none manner and showed hysteresis (once activated by a stimulus, the network maintained activity long after the stimulus was withdrawn), indicating its bistability. To make a bistable switch, another network property is also important besides positive feedback (*5*). MAPK kinase kinase activates MAPK kinase by phosphorylating two amino acid residues, and similarly, MAPK kinase activates MAPK by dual phosphorylation. These two-collision mechanisms endow the MAPK cascade with ultrasensitivity (*6*) (ultrasensitivity refers to the response of a system that is more sensitive to differences in the size of stimulus than the standard hyperbolic Michaelis-Menten response). The bistable switch behavior of the MAPK-PKC positive feedback loop arises from the combination of ultrasensitivity and positive feedback (*5*). Bistability was naturally related to synaptic plasticity because of its potential to store information.

A persistent decrease in synaptic efficacy, called long-term depression (LTD), of the parallel fiber–Purkinje cell synapse is thought to underlie some forms of cerebellar learning and memory (*7*). The biochemical process of LTD involves the following signaling pathways. Synaptic activity introduces Ca^{2+} into the cytosol, and Ca^{2+} stimulates the release of Ca^{2+} from intracellular stores in a process called regenerative Ca^{2+}-induced Ca^{2+} release (CICR). Ca^{2+} activates PLA_2 and PKC, both of which are required for LTD. PKC phosphorylates α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)–type glutamate receptors (AMPARs), leading to removal of the receptors from the cell surface through endocytosis. LTD-inducing stimuli also activate the MAPK cascade. Although these individual pathways were closely investigated, it was not clear how they would interact with one another and engage in LTD. Kuroda *et al*. (*8*) built on the work of Bhalla and Iyengar (*3*), and simulations by Kuroda *et al*. (*8*) supported the hypothesis that the bistable MAPK-PKC positive feedback loop (*3*) provided a molecular basis for cerebellar LTD. They showed that an increase in intracellular Ca^{2+} concentration after synaptic inputs led to PKC and PLA_2 activation and initiated LTD, whereas activated PKC and PLA_2 then switched on the MAPK-PKC positive feedback loop to maintain LTD for about half an hour (Fig. 1B). Furthermore, bistability of the MAPK-PKC positive feedback loop made LTD an all-or-none event according to the amount of the stimulus.

Tanaka *et al*. (*9*) partly verified this claim by showing the nonlinearity of LTD at the parallel fiber–Purkinje cell synapse. First, they showed that photolysis of a caged Ca^{2+} compound in spiny dendrites, which increased local Ca^{2+} concentration, substituted for synaptic activity and induced LTD. For better quantitative analysis, they took advantage of this form of LTD because the duration and amplitude of Ca^{2+} stimulus were relatively easy to manipulate. Ca^{2+} concentration and the average amplitude of the LTD were in a steep sigmoidal relationship. Moreover, they suggested that the response of each synapse could be all-or-none, considering noise in measurements. These findings indicated nonlinearity of the signaling network, which was consistent with the theory (*8*) that the bistable MAPK-PKC positive feedback loop plays a pivotal role in cerebellar LTD.

Tanaka and Augustine (*4*) then performed experiments to prove that LTD was dependent on the positive feedback loop. First, they demonstrated that inhibition of either the MAPK cascade or PKC abolished LTD, indicating that LTD induction required both. They next showed that activation of either the MAPK cascade or PKC resulted in activation of the other, revealing the positive feedback loop structure. Furthermore, the time window during which the PKC-MAPK positive feedback loop operates in LTD was determined to be half an hour or less from the

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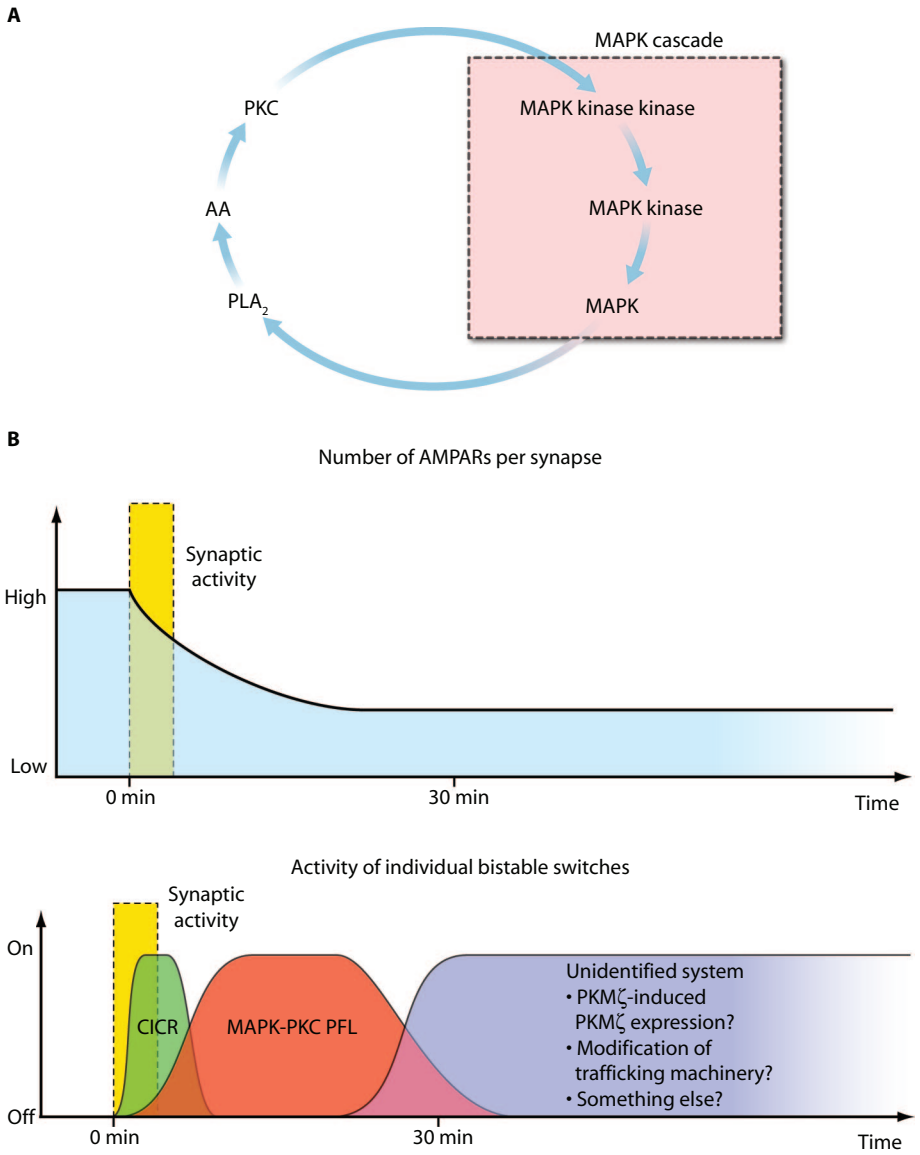
Fig. 1. Bistable switches for synaptic plasticity. (**A**) The MAPK-PKC positive feedback loop (PFL). The MAPK cascade activates PLA_2, PLA_2 activates PKC through AA production, and PKC activates the MAPK cascade, forming a PFL. (**B**) Bistable switches underlying cerebellar LTD. After synaptic activity, bistable switches are sequentially turned on, from a switch with fast kinetics, CICR, to a slower one, the MAPK-PKC PFL. Probably, the mechanism that maintains LTD after inactivation of the MAPK-PKC PFL is a bistable switch as well.



time of the LTD-inducing stimuli; a pharmacological blockade of the positive feedback loop during this period abolished or reversed LTD, whereas a later blockade did not affect its time course. Collectively, their experiments substantiated the theory of Bhalla and Iyengar (*3*) that some signal transduction pathways constitute a bistable positive feedback loop and serve as a memory, as well as the theory of Kuroda *et al*. (*8*) that the MAPK-PKC positive feedback loop plays a crucial role at a certain stage of cerebellar LTD.

The subsequent phase of LTD requires synthesis of proteins that are yet to be identified (*10*, *11*). To store information safely, its underlying mechanism should have bistable dynamics, similar to the preceding systems of the CICR (*12*) and the MAPK-PKC positive feedback loop (*3*, *4*, *8*, *9*). A set of bistable systems in which activity is transmitted sequentially from a system with fast kinetics to a system with slower kinetics might be a suitable mechanism for neuronal memory, because it seems to meet two contradictory requirements: plasticity to acquire new information flexibly, and stability to store it for a long time (Fig. 1B) (*13*–*15*). An atypical PKC isozyme, protein kinase Mζ (PKMζ) (*16*), is a candidate that may take over from the MAPK-PKC positive feedback loop (*14*). It presumably forms a bistable positive feedback loop, because its local translation is induced by PKC activity through nonlinear processes (*14*, *16*). In addition, its pivotal role in long-term memory (weeks or longer) is established in the hippocampus and neocortices (*16*).

The dendritic spine, the main site of synaptic plasticity, is so minute (~1 μm in diameter) that it contains only a limited number of molecules. Consequently, its signaling network has to operate under severe stochastic fluctuations, which will make memory formation and maintenance more difficult tasks. Now that theoretical approaches and experiments (*3*, *4*, *8*, *9*) have elucidated the deterministic behavior of average synapses, the scope should be oriented to the stochastic nature of single synapses. Advances in microscopy and visualization techniques (*17*, *18*) will enable tracking of individual molecules in living cells and provide insights into how the neuron tackles stochasticity.

The series of studies running from Bhalla and Iyengar (*3*) to Tanaka and Augustine (*4*), with Kuroda *et al*. (*8*) and Tanaka *et al*. (*9*) in between, illustrates how useful and advantageous a combination of theoretical approaches and experiments can be in deepening our understanding of cell signaling networks. It invites experimentalists to pay attention to relevant theoretical predictions and theorists to emphasize the experimental verifiability of their predictions.

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