Synaptic plasticity: **Going through phases with LTP**

Emily P. Huang

Early and late expressing components of synaptic plasticity may underlie the temporal phases of behavioral memory. New studies argue that a balance between kinase and phosphatase activity regulates the transition between different phases of synaptic plasticity and memory.

Address: Howard Hughes Medical Institute and Molecular Neurobiology Laboratory, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, California 92037, USA.

Current Biology 1998, 8:R350–R352
http://biomednet.com/elecref/09609822008R0350

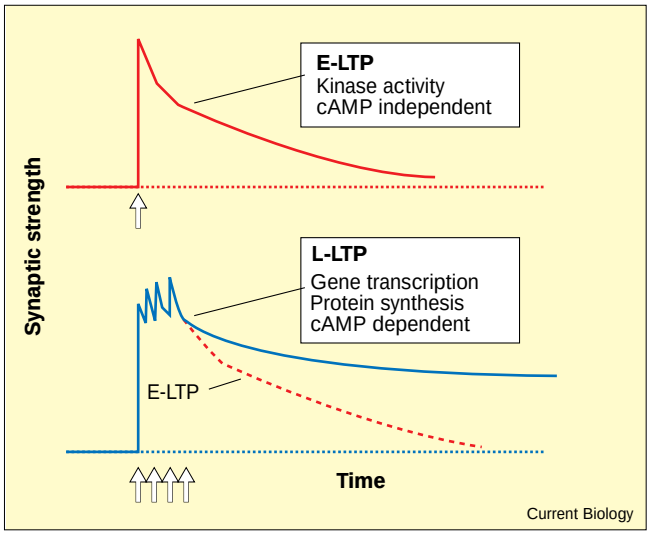
Arguably *the* holy grail of neurobiological research is to understand how the brain stores and accesses memory all the way down to the molecular level. In pursuit of this dream, researchers have expended tremendous effort in studying mechanisms of synaptic plasticity and how these mechanisms relate to behavioral memory. Long-term potentiation (LTP), an increase in synaptic strength induced by repetitive stimulation of presynaptic terminals, is a particularly well-studied form of synaptic plasticity believed to underlie memory function in the mammalian hippocampus, amygdala and other cortical brain structures [1,2].

Despite the number of studies devoted to LTP, there remains considerable controversy over its molecular mechanism. Two points of general agreement are that the initial event in LTP induction is Ca^{2+} influx through postsynaptic *N*-methyl-D-aspartate (NMDA) receptor channels, and that certain protein kinases — including the Ca^{2+}/calmodulin-dependent kinases (CaMKs), protein kinase C, and the tyrosine kinase Fyn — play an important role in LTP expression. Like puzzle pieces, however, the data gathered on LTP often refuse to fit together. One potentially complicating issue is whether LTP is divided into temporal phases, each involving different biochemical interactions. Recent studies [3,4] suggest that LTP may have several phases that can be distinguished on the basis of their induction requirements, time of expression and molecular mechanisms; furthermore, different stages of behavioral memory appear to depend on the expression of LTP phases [5].

The preservation of memories for a day or longer has been shown to depend on *de novo* gene transcription and protein synthesis. Similarly, the expression of LTP for more than a few hours *in vitro* also requires new protein synthesis [6], but the initial expression of LTP does not. On the basis of these observations, researchers [3] have suggested that LTP can be divided into at least two phases: E-LTP (early LTP) and L-LTP (late LTP). According to this scheme, E-LTP begins immediately after the LTP-inducing stimulus, lasts less than a few hours, and depends primarily on short-term kinase activity. L-LTP begins a few hours after the inducing stimulus, lasts for at least eight hours, and depends on the activation of gene transcription (see Figure 1). The induction requirements for the two phases also differ. A single train of high-frequency stimuli induces only E-LTP, and stronger stimulation protocols, such as three or four spaced trains of stimuli, are needed to recruit L-LTP. Taken all together, these physiological characteristics make E-LTP and L-LTP attractive candidate mechanisms for short-term and long-term phases of memory, respectively.

Until recently, most researchers working on LTP ignored this possible distinction, focusing on mechanisms that affect total LTP expression. Several studies, however, have tried to distinguish mechanisms that are unique to L-LTP [3,7]. Interestingly, the picture emerging from these studies evokes parallels to work on lower organisms, such as the sea slug *Aplysia* and the fruit fly *Drosophila*, in which cAMP cascades play an important role in memory

Figure 1



Early and late phases of long-term potentiation (LTP). A train of repetitive stimuli (arrows) induces an increase in synaptic strength known as LTP. A single train of stimuli induces E-LTP (red), which decays over the course of a few hours. Multiple trains also induce L-LTP (blue), which remains stable for many hours.

formation and consolidation [8]. For example, application of cAMP analogs to synapses in a hippocampal slice preparation [3] induces a slowly expressing, but long lasting, increase in synaptic strength that resembles L-LTP. Furthermore, application of cAMP-dependent kinase (PKA) inhibitors attenuates LTP expression, apparently eliminating the ability of synapses to express L-LTP. These results suggested that PKA activated by cAMP may gate the expression of L-LTP by direct, indirect or permissive activation of transcription factors. Later work indicated that 'cAMP-response element binding protein' (CREB), a transcription factor activated by elevations in Ca^{2+} and cAMP, is involved in long-term memory mechanisms in organisms from *Aplysia* to mammals [8]. Mutant mice lacking CREB display LTP that decays faster than usual and also exhibit impaired retention of spatial memories for periods longer than a few hours [7].

The emphasis on the participation of kinases in LTP expression begs the question of what role phosphatases may play in synaptic plasticity. Because kinase activity in general appears to up-regulate synaptic strength, researchers have postulated that phosphatases act to down-regulate synapses, either by promoting synaptic weakening mechanisms such as long-term depression (LTD) or by suppressing LTP [9,10]. Specific interest has devolved upon calcineurin, a Ca^{2+}/calmodulin-regulated phosphatase richly expressed in brain neurons [10], because application of calcineurin inhibitors prevents the expression of LTD in area CA1 of the hippocampus. A number of calcineurin's activities in neurons have been described [10], but of particular interest for the current discussion is its well-characterized antagonism with PKA. For example, calcineurin and PKA colocalize at neuronal membranes by binding to a common anchoring protein, AKAP79, from whence they appear to co-regulate NMDA receptors, AMPA receptors (another type of glutamate receptor) and voltage-gated ion channels [10]. Furthermore, calcineurin and PKA are able, respectively, to inhibit and activate protein phosphatase-1 (PP1). Several studies suggest this tug of war between PKA and calcineurin critically regulates the ability of the synapse to modify itself under specific conditions [11,12].

Two recent studies [4,5] expand our understanding of how kinases and phosphatases might interact to mediate different phases of LTP and memory. These papers center on the creation of transgenic mice overexpressing a truncated form of calcineurin under the CaMKIIα promoter, which limits expression to the forebrain (particularly the hippocampus). The truncated form of calcineurin lacks an autoinhibitory domain, so that the transgenic mice express 75% greater phosphatase activity than wild-type. In the first study, Winder *et al.* [4] found that hippocampal LTP was modified in the transgenic mice: whereas LTP induced by a single train of high frequency stimuli was normal, LTP induced by four spaced trains decayed significantly relative to wild-type. Thus excessive calcineurin activity impairs the expression of LTP, particularly inhibiting its long-term maintenance after a strong induction protocol.

Winder *et al.* [4] then compared LTP induced by two spaced trains of stimuli to that induced by four trains in wild-type mice. As one might expect, LTP induced by two trains decays with a time-course intermediate to that induced by one train and that induced by four trains. Previous studies had shown LTP induced by three or more trains is sensitive to both protein synthesis inhibitors and PKA inhibitors [3,6]. In contrast, Winder *et al.* [4] found that LTP induced by two trains is partially impaired by PKA inhibition but not by protein synthesis inhibition. They concluded that there is a PKA-dependent phase of LTP intermediate to E-LTP and L-LTP, which they dub I-LTP. In the transgenic mice, on the other hand, LTP induced by two trains decays faster than in wild-type mice and is unaffected by application of a PKA inhibitor, implying that both I-LTP — as defined in this study — and L-LTP are suppressed in mice with excessive calcineurin activity. From these results, the authors argue that the function of PKA and of I-LTP in general might be to overcome calcineurin inhibition of activities that lead to L-LTP expression.

In a set of complementary experiments on the same strain of calcineurin-overexpressing transgenic mice, Mansuy *et al.* [5] examined the question of whether the calcineurin-overexpressing mice display memory-related behavioral deficits. The authors trained the mice on the 'Barnes maze', where the mouse has to navigate a brightly lit, circular maze to find an escape tunnel. Performance on this spatial memory task is known to depend critically on hippocampal function. When trained once daily on this task and tested over time for their ability to learn the tunnel location, the transgenic mice were found to perform poorly compared to wild-type mice, indicating that they have a deficit in spatial memory. The transgenic mice are not absolutely impaired, however: when trained more intensively — four times a day — their performance deficit relative to wild-type mice disappears.

Mansuy *et al.* [5] investigated the memory deficit of the calcineurin-overexpressing mice further by testing them on a different memory task that also depends on hippocampal function. In this task, the mice are initially exposed to two objects for a limited time. Later, the mice are exposed to a third, novel object along with one of the original two; the relative amount of time the mouse spends exploring the novel object is used as a measure of its object recognition memory. The advantage of this task is that one can evaluate the time-course of memory retention after one initial training session. Mansuy *et al.* [5]

found that the transgenic mice perform normally on this task at 30 minutes after training, but are significantly impaired at 24 hours after training. In all, these results imply that calcineurin-overexpressing mice have a deficit in long-term memory consolidation that correlates with their deficit in late phase LTP.

Although these studies do suggest that active calcineurin can negatively regulate LTP and memory, particularly on a time-course associated with long-term consolidation, there are some reservations. First, the level of LTP expression from one experiment to the next tends to be highly variable, so conditions of partial LTP inhibition must be interpreted cautiously. This problem is especially acute when evaluating effects on different phases of LTP, which may turn 'on' and 'off' with uncertain timing and which probably share some mechanisms. Second, the studies examined the effects of overexpressing a calcineurin transgene; more work must be done to see whether endogenous calcineurin functions in a similar manner. Finally, the proposed interaction between calcineurin and PKA in gating the late phases of LTP and memory must be confirmed by identifying specific downstream targets that mediate the effect.

Nonetheless, these studies reinforce the link between long-term memory consolidation and the ability to express long-lasting LTP. They also highlight the importance of treating LTP as a dynamic phenomenon that may be modulated by many factors, including time. This point was recently illustrated in a study comparing synaptic transmission during two different phases of LTP. Bolshakov *et al.* [13] induced LTP at putative single synapses in hippocampal slices and compared the properties of transmission during E-LTP and L-LTP. Their results indicate that the increased synaptic strength observed in the two LTP phases may be based on different mechanisms. More specifically, they imply that E-LTP involves a change in presynaptic transmitter release, but that in L-LTP both presynaptic and postsynaptic properties are modified (but see [14]). Bolshakov *et al.* [13] suggest that some of the previous conflict over the site of LTP expression [14,15] might arise from confusing E-LTP and L-LTP, which may arise at different times under different conditions. So for all those who have been confused and bemused by LTP over the years, it might be time to punch the clock.

References

1. Tsien JZ, Huerta PT, Tonegawa S: The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 1996, 87:1327-1338.
2. Rogan MT, Staubli UV, LeDoux JE: Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 1997, 390:604-607.
3. Frey U, Huang YY, Kandel ER: Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 1993, 260:1661-1664.
4. Winder DG, Mansuy IM, Osman M, Moallem TM, Kandel ER: Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* 1998, 92:25-37.
5. Mansuy IM, Mayford M, Jacob B, Kandel ER, Bach ME: Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. *Cell* 1998, 92:39-49.
6. Frey U, Krug M, Reymann KG, Matthies H: Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region ***in vitro***. *Brain Res* 1988, 452:57-65.
7. Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ: Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* 1994, 79:59-68.
8. Bailey CH, Bartsch D, Kandel ER: Toward a molecular definition of long-term memory storage. *Proc Natl Acac Sci USA* 1996, 93:13445-13452.
9. Mulkey RM, Endo S, Shenolikar S, Malenka RC: Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* 1994, 369:486-488.
10. Yakel JL: Calcineurin regulation of synaptic function: from ion channels to transmitter release and gene transcription. *Trends Pharmacol Sci* 1997, 18:124-134.
11. Blitzer RD, Wong T, Nouranifar R, Iyengar R, Landau EM: Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. *Neuron* 1995, 15:1403-1414.
12. Thomas MJ, Moody TD, Makhinson M, O'Dell TJ: Activity-dependent β-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. *Neuron* 1996, 17:475-482.
13. Bolshakov VY, Golan H, Kandel ER, Siegelbaum SA: Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron* 1997, 19:635-651.
14. Liao D, Hessler NA, Malinow R: Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* 1995, 375:400-404.
15. Stevens CF, Wang Y: Changes in reliability of synaptic function as a mechanism for plasticity. *Nature* 1994, 371:704-707.