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Mechanism for a Sliding Synaptic Modification Threshold

Minireview

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Long-term potentiation (LTP) is a long-lasting increase in synaptic effectiveness that results from high frequency stimulation (HFS) of certain excitatory synapses in hippocampus and neocortex (reviewed by Bliss and Collingridge, 1993). Because it is induced by brief episodes of electrical stimulation that resemble patterns observed in the brains of behaving animals, and because it can be very long-lasting, LTP has attracted a great deal of interest as a possible synaptic mechanism for learning and memory. In addition to this role, it has been suggested that the properties of LTP are also well-suited to account for aspects of experience-dependent development. LTP results when presynaptic activity coincides with strong postsynaptic depolarization beyond a threshold value. Thus, the development of binocularly responsive neurons in the visual cortex, for example, could be accounted for by associative LTP caused by the simultaneous activation of converging inputs from the two eyes.

In addition to an LTP-like mechanism, it has been recognized for some time that, to account for developmental plasticity in the cortex, there must be a mechanism for activity-dependent decreases in synaptic strength. For example, during a critical period of early postnatal life, depriving an eye of visual patterns leads to an activity-dependent decrease in the effectiveness of deprived synapses in visual cortex. Indeed, the activity-dependent pruning of connections is a very prominent feature of postnatal development throughout the nervous system. Thus, developmental neurobiologists have sought an experimental model for use-dependent decreases in synaptic strength in the CNS. Here, theoretical analysis of developmental plasticity has been of considerable value. According to the Bienenstock–Cooper–Munro (BCM) theory developed to account for aspects of visual cortical development, long-term depression (LTD) should result when input activity consistently fails to activate postsynaptic neurons beyond the threshold for LTP (Bienenstock et al., 1982; Bear et al., 1987). This insight inspired the attempt to produce LTD with repetitive low frequency stimulation (LFS; Dudek and Bear, 1992). LFS-induced LTD is now a well-established model of synaptic plasticity in slices of hippocampus and neocortex (reviewed by Bear and Malenka, 1994).

In the CA1 region of hippocampus and in layer III of visual cortex, LFS of excitatory afferents produces LTD, and HFS produces LTP (Kirkwood et al., 1993). Both forms of synaptic plasticity depend on N-methyl-D-aspartate (NMDA) receptor activation and postsynaptic Ca^{2+} entry. Available data support a model in which the state of correlation of pre- and postsynaptic activity is converted by the voltage-dependent NMDA receptor channel into a graded postsynaptic Ca^{2+} signal: Ca^{2+} triggers LTD at low concentrations and triggers LTP at high concentrations (Bear and Malenka, 1994). The smooth transition from net LTD to net LTP may be demonstrated by systematically varying the frequency of conditioning stimulation. Figure 1 shows such a frequency–response function for rat CA1 and compares it with the modification function of the BCM theory. If the effects of varying stimulation frequency are explained by different values of postsynaptic response during the conditioning stimulation, for which there is good evidence, it can be seen that the data are in striking agreement with assumptions of this theory.

Bienenstock et al. (1982) called the critical level of postsynaptic response at which the sign of the synaptic modification reverses from negative (LTD) to positive (LTP) the modification threshold (θ_m). An additional key assumption of the BCM theory is that the value of θ_m is not fixed, but rather slides as a function of the history of postsynaptic activity. Thus, after a period of increased activity, θ_m slides to the right, promoting synaptic depression; after a period of decreased activity, θ_m slides to the left, promoting synaptic potentiation. This feature is required for the pattern of synaptic weights to reach a stable state.

The association of the experimentally derived LTD/P crossover point ($\theta_{LTD/P}$) with a critical level of NMDA receptor activation and Ca^{2+} entry led to the suggestion of a number of possible mechanisms for a sliding threshold, but until recently data for any of these have been lacking. Now Mark Mayford, Eric Kandel, and colleagues (Mayford et al., 1995; Bach et al., 1995) have provided evidence for a novel molecular mechanism. I shall briefly review these findings and then return to the question of their broader significance.

Prior Activity May Shift the LTD/P Crossover Point

LFS-induced LTD is of relatively small magnitude in adult CA1. However, following establishment of LTP with HFS, conditioning stimulation at the same low frequency (typically 1 Hz) results in far greater depression (e.g., Fujii et al., 1991). Perhaps 1 Hz stimulation, in addition to producing LTD de novo, also causes "depotentiation" by interfering with the mechanisms of LTP consolidation. Alternatively, a tetanus used to generate LTP could alter the LTD/P modification function so that LTD is more likely after 1 Hz stimulation. The latter interpretation is supported by evidence that a tetanus that fails to produce lasting LTP can still promote LTD following 1 Hz stimulation (Wexler and Stanton, 1993; Wagner and Alger, 1995). Moreover, prior tetanic stimulation can also inhibit subsequent induction of LTP (Huang et al., 1992). These data suggest that HFS shifts $\theta_{LTD/P}$ to the right.

By using a stimulation frequency near the LTD/P crossover point, Mayford et al. have provided additional evidence that a prior tetanus alters the value of $\theta_{LTD/P}$. In adult mouse CA1, they show that 5 Hz stimulation (900 pulses)

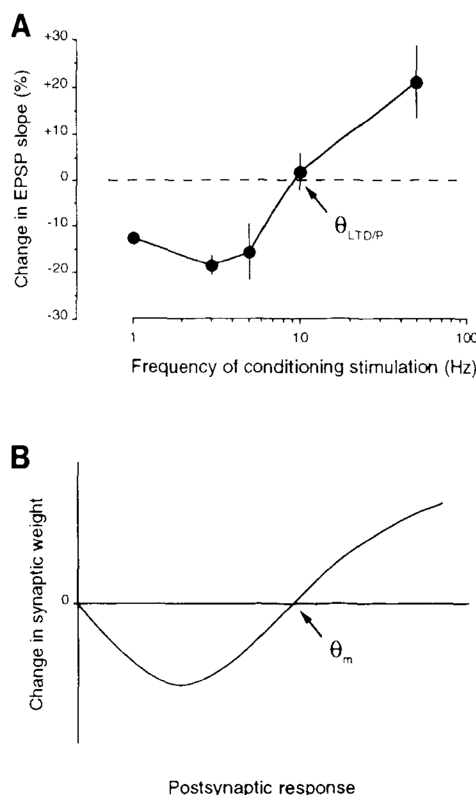

Figure 1. Comparison of Theory and Experiment

(A) The mean (± SEM) effect of 900 pulses of conditioning stimulation delivered at various frequencies to the Schaffer collaterals on the synaptic response measured in rat CA1 30 min postconditioning (replotted from Dudek and Bear, 1992).

(B) The BCM theory. The weights of active synapses are assumed to modify according to this function of the postsynaptic response. At a critical value of postsynaptic response, called the modification threshold (θ_m), the function changes sign from negative to positive. The value of θ_m is not fixed, but varies continuously as a function of the average postsynaptic response.

of the Schaffer collaterals results in a modest synaptic potentiation (unlike the rat data shown in Figure 1, where 5 Hz produces depression). However, the same 5 Hz stimulation causes robust depression when it is delivered 20 min after HFS (which also causes LTP). A complication in interpreting this result is that HFS altered both prior postsynaptic activity and synaptic weight. If the modest net LTP observed after 5 Hz stimulation normally reflects potentiation of some synapses and depression of others, then 5 Hz delivered after LTP has been saturated (after HFS) would be expected to unmask LTD. Thus, it would be desirable to have similar data from experiments in which HFS did not also cause LTP. Nonetheless, the change in sign of the synaptic modification resulting from a single stimulation frequency is consistent with the hypothesis that $\theta_{LTD/P}$ moved as a consequence of the prior stimulation.

If $\theta_{LTD/P}$ has indeed shifted, what is the mechanism? One consequence of postsynaptic depolarization and Ca^{2+} entry is activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a major postsynaptic protein (reviewed by Hanson and Schulman, 1992). Ca^{2+}/calmodulin activation of CaMKII leads to autophosphorylation at Thr-286 of the α subunit, and this has two interesting consequences: it switches the enzyme to an autonomous (Ca^{2+}-independent) state, and it increases the affinity of the enzyme for calmodulin by 1000-fold (Hanson and Schulman, 1992). Both changes allow the catalytic activity of the enzyme to persist long after the Ca^{2+} concentration has returned to basal levels. In hippocampus it has now been shown that HFS of the Schaffer collaterals can increase Ca^{2+}-independent CaMKII activity for at least an hour (Fukunaga et al., 1993). It has been suggested that the switching of CaMKII to the autonomous state is how LTP is maintained (reviewed by Lisman, 1994). However, it is also possible that autophosphorylation of CaMKII holds the short-term memory that shifts the modification threshold.

Transgenic Mice Expressing Autonomous CaMKII Have a Shifted LTD/P Crossover Point

To examine the functional consequences of increasing the amount of autonomous CaMKII, Mayford et al. introduced into mice a transgene for the α subunit of CaMKII with a mutation at codon 286, replacing Thr with Asp. Like the autophosphorylated wild-type enzyme, CaMKII–Asp-286 is active and Ca^{2+}-independent. Although only 10%–15% of the CaMKII expressed in the transgenic mice was CaMKII–Asp-286, hippocampal extracts showed double the level of Ca^{2+}-independent CaMKII activity of wild-type animals.

What are the functional consequences of increased autonomous CaMKII activity in the transgenic animals? Surprisingly, LTP evoked by HFS of the Schaffer collaterals was indistinguishable in CA1 of transgenic and wild-type animals. However, a marked difference was noted when lower stimulation frequencies were used. Stimulation at 5 Hz, which, as mentioned previously, produces a slight potentiation in CA1 of adult wild-type mice, caused significant depression in the transgenic mice. Thus, the value of $\theta_{LTD/P}$ is shifted to the right in mice expressing high levels of autonomous CaMKII activity.

Is the shift of $\theta_{LTD/P}$ in transgenic animals related to that which occurs after a prior tetanus in the wild type? To examine this possibility, HFS was delivered to the Schaffer collaterals (and LTP was produced) in both transgenic and wild-type animals, and then the effects of subsequent 5 Hz stimulation were compared. In this case, there was no difference observed in the magnitude of the depression caused by 5 Hz stimulation in wild-type and transgenic animals. Thus, expression of the transgene occludes the effects of HFS on $\theta_{LTD/P}$. This suggests that the shifting of $\theta_{LTD/P}$ by HFS in wild-type animals is due to the elevation of autonomous CaMKII activity. In addition, the data suggest that in adult transgenic animals $\theta_{LTD/P}$ is fixed as well as shifted to the right.

Autonomous CaMKII Activity and LTD during Early Postnatal Development

LTD is more robust early in postnatal life and declines with increasing age (Dudek and Bear, 1993). There is also a developmental decline in autonomous CaMKII activity

(Molloy and Kennedy, 1991). Are these two developmental changes related? Mayford et al. found that immature (3–4 weeks) wild-type animals exhibited the same robust LTD after 1 Hz stimulation as immature transgenic animals, even though the autonomous CaMKII activity in the wild type was half the mutant value. This result suggests that the absolute magnitude of LTD (assessed using 1 Hz stimulation) may be unrelated to the amount of autonomous CaMKII.

The level of autonomous CaMKII activity appears to be more closely related to the value of $\theta_{LTD/P}$. In adult wild-type mice, 5 Hz stimulation yields a modest LTP, but in young animals it yields significant LTD. Analysis of data from young and mature wild-type and mutant animals revealed a striking correlation: the greater the autonomous CaMKII activity, the greater the magnitude of the LTD caused by 5 Hz stimulation. Because 5 Hz stimulation is near $\theta_{LTD/P}$, this finding suggests that the percentage of autonomous CaMKII activity is positively correlated with the value of $\theta_{LTD/P}$.

Transgenic Mice Expressing Autonomous CaMKII Exhibit a Selective Deficit in Hippocampal-Dependent Memory as Adults

Bach et al. (1995) examined whether the mutant mice with altered synaptic plasticity exhibit deficits in a battery of memory tests. Mutants expressing autonomous CaMKII showed no deficit in their ability to associate an aversive stimulus with a novel environment (contextual fear conditioning). However, a profound deficit was noted in a task that requires animals to learn to navigate to a specific location using only spatial cues (the Barnes circular maze). This dissociation is interesting in light of the fact that both types of memory are sensitive to lesions of the hippocampus. The fact that these two types of memory can be dissected genetically suggests that they may have very different neural substrates.

The spatial memory deficit does not correlate with a change in HFS-induced LTP in CA1, which is normal in the mutants. The authors point out that this memory deficit might be a result of the observed deficit in LTP produced by 5 Hz (θ frequency) stimulation. Equally plausible, however, is that this is a behavioral consequence of shifting and fixing $\theta_{LTD/P}$, which, in theory, would disrupt memory formation. In any case, the important message is that, when it comes to making the connection between synaptic plasticity and memory, we are still much like the blind man and the elephant. Understanding the nature of the beast will require multidimensional analyses. It is clearly too simplistic to associate LTP with hippocampal-dependent memory formation; LTD must also be considered, as well as the functions that relate synaptic activity to synaptic change.

How Does CaMKII Autophosphorylation Slide the Modification Threshold?

Mayford et al. make a compelling argument that the level of autonomous CaMKII activity sets the value of the LTD/P modification threshold. According to this hypothesis, the value of $\theta_{LTD/P}$ is raised when a surge of Ca^{2+} results in autophosphorylation of the kinase (Figure 2). This new

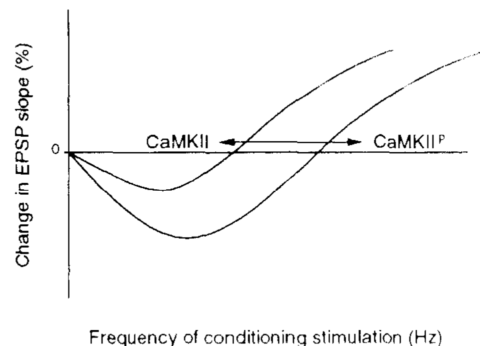

Figure 2. Molecular Mechanism for a Sliding Modification Threshold

value of $\theta_{LTD/P}$ can persist as long as CaMKII remains Ca^{2+} independent, which, in vitro, can last for minutes to hours, possibly longer, depending on the conditions (e.g., Fukunaga et al., 1993).

How might CaMKII regulate $\theta_{LTD/P}$? Induction of both LTD and LTP requires postsynaptic Ca^{2+} entry and the binding of Ca^{2+} to calmodulin. Low levels of Ca^{2+}/calmodulin are believed to trigger LTD by activating a network of protein phosphatases (Mulkey et al., 1994), and high levels of Ca^{2+}/calmodulin are believed to trigger LTP by activating a network of protein kinases (Malenka et al., 1989; Malinow et al., 1989). CaMKII autophosphorylated at Thr-286 has a higher affinity for calmodulin than any other known postsynaptic protein (Hanson and Schulman, 1992). Calmodulin trapped on autophosphorylated CaMKII would reduce the free calmodulin in the postsynaptic dendritic spine and would also act as a Ca^{2+} sink by competing with free calmodulin. Computational models have shown that regulation of Ca^{2+} buffering in a dendritic spine could account for a sliding θ_m (Gold and Bear, 1994). Of course, for the calmodulin-trapping model to account for the data of Mayford et al., it must be shown that calmodulin affinity is also increased in the CaMKII–Asp-286 mutant. Another possibility is that calmodulin binding is actually *blocked* by the Ca^{2+}-independent autophosphorylation at Thr-305. Phosphorylation of this site locks the enzyme into a state of submaximal activity and renders it insensitive to subsequent elevations in Ca^{2+} (Hanson and Schulman, 1992).

One can imagine numerous other possible mechanisms whereby CaMKII could regulate $\theta_{LTD/P}$. More work will be required to understand the downstream consequences of autonomous CaMKII activity on regulating synaptic plasticity.

Synaptic Plasticity and Metaplasticity

Previous studies implicated CaMKII activation as a necessary step in the induction of LTP. Thus, LTP is blocked by inhibitors of CaMKII catalytic activity (Malenka et al., 1989; Malinow et al., 1989) and is greatly diminished in mutant mice lacking the CaMKII α subunit (Silva et al., 1992). Moreover, postsynaptic introduction of a constituitively active CaMKII fragment increases synaptic responses and occludes LTP (Pettit et al., 1994). A role for CaMKII in triggering a change in synaptic strength, how-

ever, does not exclude a role for CaMKII autophosphorylation in regulating the synaptic modification function.

Mayford et al. provide evidence that elevating the Ca^{2+}-independent activity of CaMKII alters $\theta_{LTD/P}$. The BCM theory gives us a valuable framework for understanding the possible functional significance of this change, but it is premature to conclude that CaMKII autophosphorylation is the basis for the sliding modification threshold of the BCM theory. There are two theoretical requirements for the BCM modification threshold, θ_m: its value must be a function of the history of the entire cell's (or relevant computational unit's) postsynaptic activity, and its value must be the same at all modifiable synapses on this cell. So far, the examples of activity-dependent shifts in $\theta_{LTD/P}$ have been homosynaptic, restricted only to the stimulated synapses. The properties of the BCM sliding threshold would be satisfied only if cellular activity and voltage-gated Ca^{2+} entry into dendrites caused widespread CaMKII autophosphorylation in spines (assuming this is the site where the LTD/P crossover point is regulated by CaMKII). More work is required to prove the existence of a sliding synaptic modification threshold of the sort envisaged by Bienenstock et al. (1982) and to identify its mechanism.

It has become increasingly clear in recent years that neural activity, which by itself produces little or no change in synaptic effectiveness, can set in motion changes that alter the properties of synaptic plasticity. Activity-dependent plasticity of synaptic plasticity, or metaplasticity (a term suggested by W. C. Abraham), may play an important role in the modification of neural networks, both in development and during learning. The studies of Mayford et al. suggest a molecular mechanism for one form of metaplasticity. However, autophosphorylation of CaMKII is likely to be only one in a class of activity-dependent mechanisms that alter the properties of synaptic plasticity. Other mechanisms for metaplasticity are likely to include activity-dependent changes in inhibition (Wagner and Alger, 1995), NMDA receptor properties (Carmignoto and Vicini, 1992), dendritic spine morphology (Gold and Bear, 1994), and expression of genes, including that for αCaMKII (Neve and Bear, 1989). With LTD and LTP, we have the tools to study metaplasticity; with genetic manipulations in mice, we now have the opportunity to understand its behavioral significance.

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