A molecular mechanism for metaplasticity

The results of expressing a constitutive form of a prominent synaptic kinase in transgenic mice suggest how there can be a sliding threshold for synapse modification, an important element in some learning theories.

Experimental neuroscientists who work on synaptic plasticity usually spend their time down at the molecular level investigating basic mechanisms, whereas theoreticians who inhabit the abstract world of neural network models hardly ever consider biochemical events. So it is rare, but extremely gratifying, when an advance on one front actually clarifies the picture on another. A case in point is a set of studies from Mayford, Kandel and colleagues, published recently in *Cell* [1,2]. These papers describe the functional and behavioral effects of expressing in transgenic mice a constitutively active, mutant form of a key synaptic enzyme, the α isoform of calcium–calmodulin-dependent kinase II (CaMKII) [3,4].

CaMKII is known to be important for stable changes in synaptic strength — both hippocampal long-term potentiation (LTP) and long-term depression (LTD) [5–8]. Mayford *et al.* [1] introduced into mice a transgene encoding an altered form of CaMKII —CaMKII(T286D), in which threonine 286 is replaced by aspartic acid to mimic the effect of autophosphorylation; this change makes the enzyme constitutively active even in the absence of calcium or calmodulin [3,4]. When the basic synaptic physiology of these transgenic lines was tested, the resting or basal synaptic strength appeared normal, and synaptic plasticity appeared not to have been directly induced. Strikingly, however, a fascinating change was seen to have occurred in the rules governing plasticity. Synaptic stimulation at a frequency that would have induced a slight LTP in wild-type mice instead induced robust LTD in the mutants. Furthermore, the amount of depression induced correlated well with the level of calcium-independent CaMKII activity achieved. These results strongly indicate that the genetic manipulation specifically altered the decision-making mechanisms for plasticity — exemplifying a phenomenon that has long interested neural theorists [9], and that has recently been termed 'metaplasticity' (W.C. Abraham, personal communication, and [10]).

A simple example of metaplasticity is shown in Figure 1. Consider a synaptic pathway that gives a fixed response (shown as an excitatory postsynaptic potential in Fig. 1a) under basal conditions, and that undergoes LTP when subjected to an inducing stimulus pattern (⋆). Now suppose that, after a special intervention (†), the same stimulus pattern (⋆) instead causes LTD. The changeover from one form of synaptic strength change to another is a kind of plasticity of plasticity, or 'metaplasticity' (W.C. Abraham, personal communication, and [10]). Notice that the metaplastic change might be tricky to detect — the obvious functional properties of the neurons need not change at all. Instead of seeing a change in synaptic strength itself, the experimenter might only see a change in the rules used by the neurons to change synaptic strength. For example, hippocampal or cortical synapses appear to implement a well-defined function for altering their strength in response to different inducing frequencies: lower stimulation frequencies induce LTD, and higher frequencies produce LTP [11]. Metaplasticity might involve a change in the threshold frequency, θ_m, that separates net LTD from net LTP (Fig. 1b). A situation in

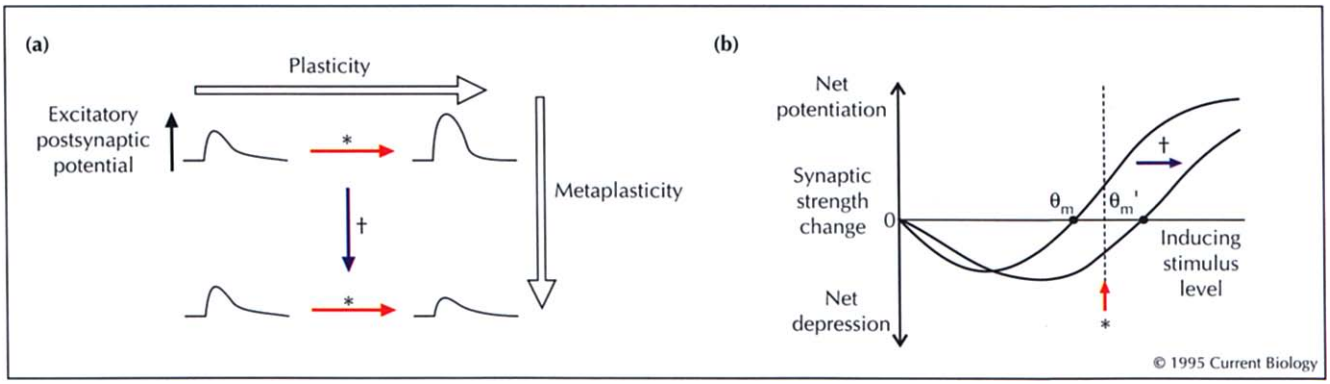


Fig. 1. (a) Plasticity and metaplasticity. Plasticity is the change in synaptic strength produced by a standard inducing stimulus (*); metaplasticity is the altered impact of * following another stimulus (†) (see text for details). **(b)** A good way of depicting plasticity rules is to plot the changes in synaptic strength as a function of the inducing stimulus level. The frequency requirements of LTD and LTP overlap in such a way as to produce a threshold θ_m, above which net potentiation takes place and below which net depression occurs (see Box 1). One type of metaplasticity, induced by † in (a), can be expressed as a change in the input–output relationship that results in a shift in the threshold θ_m to θ_m'. Here * marks the inducing stimulus used in (a).

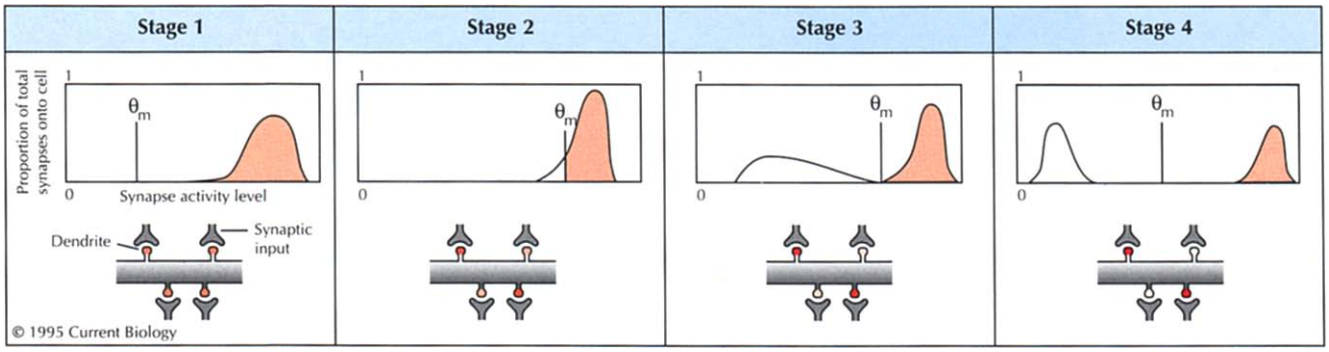


Fig. 2. How metaplasticity can influence the evolution of synaptic plasticity. The graphs (top) show the distribution of synaptic responses as a neuron evolves through four stages; corresponding views of the development of four synapses randomly selected from these distributions are also shown (bottom; changes in the intensity of red shading of the synaptic inputs represents changes in their strength). Suppose that during network development, or in response to some environmental change, the activity of synaptic inputs onto a neuron increases dramatically, so that all of the synapses are excited at levels above the plasticity threshold θ_m (stage **1**). If the neuron were simply to continue implementing a plasticity rule of the kind depicted in Fig. 1b, the synapses would continue to strengthen, and eventually saturate at some upper limit. At this point all of the synapses would have similar effects and the neuron would essentially fire continuously without regard to the input pattern. Not only could this be dangerous to the neuron, but the firing of the neuron would not carry very much information about the pattern of input activity! To circumvent this problem, a well-designed neuron might implement a rule for detecting the aggregate synaptic input and raise the threshold θ_m (enhancing LTD at the expense of LTP). Some synapses would now fall below the new θ_m (stage **2**), and consequently weaken (stage **3**). Eventually a stable state would be reached, with some strong and some weak synapses (stage **4**). Now only a subset of the possible patterns of synaptic activity would result in spike firing in the postsynaptic cell. As a kind of metaplasticity, the sliding threshold is therefore useful for explaining how neurons which are not optimally tuned initially might develop selectivity for patterns of inputs. Note that LTD and LTP fill roles of equal importance in this formalism; LTD is not simply a means of erasing prior LTP to avoid saturation of synapses at a maximal level.

which this sort of change may be very important for neural processing is illustrated in Figure 2. Note that, although we have focused on synaptic modification, changes in dendritic integration or spike threshold would also suffice to alter the way that decisions about neuronal firing are made.

Can metaplasticity actually occur in the brain? In recent years, a number of groups have reported synaptic phenomena that can clearly be classified as metaplasticity [12–14], and some have provided initial clues as to its mechanism. Huang *et al.* [12] showed that synaptic activity which does not itself cause a stable synaptic strength change can prevent later LTP, by a mechanism requiring calcium influx through *N*-methyl-D-aspartic acid (NMDA) receptors. In their hands, the metaplasticity was synapse-specific and lasted less than an hour. Similarly, O'Dell and Kandel [13] observed a transient prevention of later LTP by prior non-plasticity-inducing synaptic activity, and went on to show that this metaplasticity required activation of protein phosphatases. Other studies have suggested the involvement of a protein kinase C in this kind of metaplasticity [15,16].

How might metaplasticity be stored in a neuron? Covalent modification of proteins is a plausible biochemical mechanism. One of the first possibilities to be considered was phosphorylation of the NMDA receptor itself [17]. If this modification were to reduce calcium entry through activated NMDA receptors, synaptic stimuli that normally induce LTP might fail to do so, as in the experiments of Huang *et al.* [12] and O'Dell and Kandel [13]. On the other hand, Mayford *et al.* [1] considered that the storage of metaplasticity might involve an intracellular signalling enzyme downstream of the NMDA receptor [17] — the α subunit of CaMKII. Mayford *et al.* [1] analyzed two lines of transgenic mice expressing different copy numbers of the gene for the calcium-independent mutant enzyme αCaMKII(T286D). Biochemical experiments using hippocampal extracts showed that basal calcium-independent activity of the enzyme was appropriately elevated in the mutants. The central finding was that this increase in calcium-independent CaMKII activity in the transgenic mice was sufficient to induce metaplasticity, favoring LTD over LTP (as in Fig. 1). The authors went on to show that the variations in constitutively active CaMKII levels might actually regulate decision-making about plasticity under physiological circumstances, during brain development or following a bout of intense synaptic activity.

First, consistent with previous work on young rats [18], Mayford *et al.* [1] showed that, in wild-type mice, basal calcium-independent CaMKII activity was higher in young animals than in adults, in parallel with the prominence of LTD in young animals. They also found that the quantitative relationship between enzymatic activity and the propensity for LTD was the same for transgenic as for wild-type mice; no more LTD was observed in young transgenic animals than in either young wild-type or adult transgenic animals. Thus, youth and αCaMKII(T286D) transgene expression appear to occlude each other's effect on synaptic plasticity.

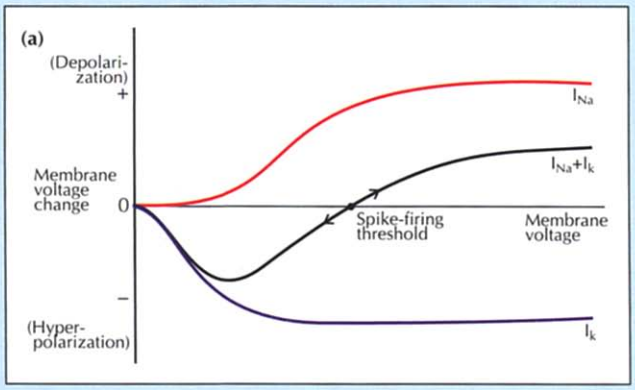
Next, experiments were conducted to test the effects of combining high-frequency stimulation and expression of αCaMKII(T286D). It was already known that high-frequency stimulation can produce long-term increases in

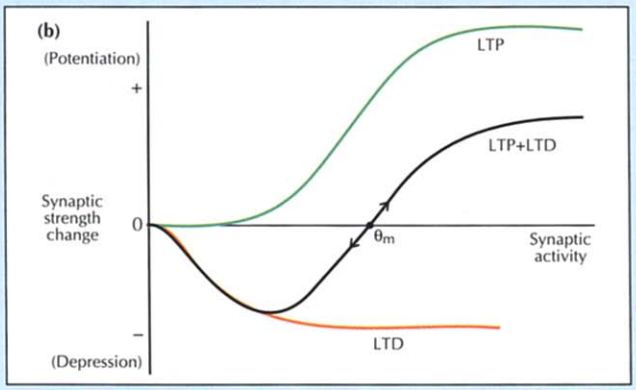
Establishing and shifting a threshold

How can a sharp threshold be achieved? Realistic biochemical processes do not show an absolute threshold, but are gradually augmented by increasing input. However, two processes that are largely overlapping but of opposite sign can combine together to produce a net effect that changes sign at a crossover point. This point will represent a threshold — an unstable point — if small perturbations on either side lead to runaway deviations from that point.

Examples of this are the ignition point of a bomb or the threshold for nerve excitation. In the case of excitable tissues, the overlapping processes are an inward current carried by Na^+ channels and an outward current carried by K^+ channels. Activation of these channels is a graded function of membrane depolarization but, in combination, they can produce a sharp threshold (**a**). In the case of plasticity control, the overlapping processes correspond to the forces underlying potentiation (LTP) and depression (LTD) of synaptic strength. The activation of both these processes is also a graded function of synaptic stimulation strength and, again, in combination a threshold is produced (**b**). The diagrams are intended to convey system dynamics, and correspond to phase-plane diagrams in which the *x* axis represents the state of the system, and the *y* axis represents the derivative of the state.The idea of a sliding threshold is not limited to metaplasticity — the British physiologist A.V. Hill proposed just this concept as a way of accounting for accommodation of spike firing in nerves subjected to slowly rising stimuli. Depolarization was proposed slowly to increase the threshold potential, such that weakly rising stimuli could produce strong depolarization, yet fail to elicit spike firing.

What mechanisms could allow a threshold to slide? It may be helpful to consider how the threshold for spike production is displaced. In a mechanism uncovered by Hodgkin and Huxley, the threshold for spike firing is modified by inactivation of Na^+ channels or activation of K^+ channels. These processes are particularly interesting, as they may persist without a net change in membrane potential. This offers a possible analogy with modifications of the threshold for synaptic plasticity (θ_m) — metaplasticity — that do not involve a change in synaptic strength as such. Note that shifts in threshold are not inherently unidirectional, but can go in either direction, as a result of changes in either the magnitude of the opposing processes or their dependence on stimulus strength. Indeed, like changes in the threshold for spike firing, the modification in θ_m can shift in favor of LTP [15,30] or in favor of LTD [1].





hippocampal calcium-independent CaMKII activity [19,20], while greatly enhancing subsequent LTD (or 'depotentiation') [14,21]. Mayford *et al.* [1] found that αCaMKII(T286D) expression in transgenic mice occludes the effect of such high-frequency stimulation on the propensity for LTD, in that no more LTD was observed under both conditions than with either alone.

The overall conclusion is that the effects on LTD of αCaMKII(T286D) expression, youth and high-frequency stimulation are all mutually occlusive. Under all of these conditions, there appears to be a simple and unique relationship between the degree of autonomous CaMKII activity and the propensity for LTD. Taken together, the data constitute strong evidence that long-term modification of CaMKII activity is directly involved in the metaplasticity associated with development and with synaptic input.

In a companion paper, Bach, Mayford and colleagues [2] report the results of testing the transgenic mice for behavioral abnormalities in two hippocampus-dependent learning tasks. Strikingly, the transgenic mice showed deficits in spatial memory, as tested in a Barnes maze. Their contextual fear conditioning, however, was normal, indicating that some types of hippocampus-dependent memory are intact. These results are consistent with the view that metaplasticity is important in spatial memory, but also leave open other possibilities involving developmental effects of the transgene.

As intriguing as all this work is, we still do not know how the plasticity threshold is shifted (see box). CaMKII has at least two possible actions, as a multifunctional kinase, and, given its high concentration in neurons, as a regulatable calmodulin sink. This latter property becomes prominent upon autophosphorylation, which increases the kinase's affinity for calmodulin 400-fold [22]. Perhaps it is the kinase activity of CaMKII that is involved in the weakening of NMDA receptor responses following a bout of low-frequency stimulation [23]. The resulting reduction of calcium influx would be expected to tip the balance away from LTP towards LTD at any frequency. It is unclear whether such changes in NMDA receptor properties can be induced, directly or indirectly, by CaMKII [24]. However, mice lacking αCaMKII show brain hyperexcitability, consistent with a role for CaMKII in depressing overall neuronal activity [25].

In another possibility that Mayford *et al.* [1] considered, the calmodulin-buffering properties of autophosphorylated CaMKII [22] come into play. Presuming that CaMKII(T286D) traps calmodulin as efficiently as the autophosphorylated kinase, it might sequester synaptically-generated calcium–calmodulin, and thereby reduce the effects of calcium–calmodulin on other enzymes. This idea could be tested by looking at metaplasticity in transgenic mice expressing the calcium–calmodulin-binding peptide of CaMKII in the absence of any of its other regulatory or catalytic domains.

Mayford *et al.* [1] found that expression of constitutively active CaMKII in transgenic mice caused neither direct enhancement of synaptic strength nor occlusion of LTP. This is contrary to recent work from Pettit *et al.* [8], who introduced the gene for a truncated, constitutively active form of αCaMKII into hippocampal slices by means of vaccinia virus infection. Remarkably, the virally-expressed truncated αCaMKII by itself caused a potentiation of synaptic strength comparable to, and occlusive with, LTP. This discrepancy between the two reports might stem from a side-effect of the viral infection at 35 °C, or long-term adaptation to a chronic elevation of calcium–calmodulin-independent CaMKII activity in the transgenic mice

A more interesting possibility is that the discrepancy between the results of Mayford *et al.* [1] and those of Pettit *et al.* [8] arises from differences between the active forms of CaMKII that were introduced. The T286D mutant that Mayford *et al.* used [1] would be able to form heteromultimers with wild-type α and β subunits, and would find its normal cellular location. But the molecule introduced by vaccinia infection was truncated at residue 290, and would therefore be unable to form heteromultimers or to soak up calcium–calmodulin. For these reasons, the use of truncated forms of the enzyme might emaphasize different aspects of CaMKII function.

Returning to the issue of metaplasticity, the selective alteration to the threshold between net depression and potentiation [1,2] may be only one of a number of forms of metaplasticity. Based on a reading of the literature, at least two types of metaplasticity might eventually be differentiated on the basis of their temporal properties, degree of synapse specificity and, perhaps, biochemical mechanism. In one type, changes in LTD/LTP induction would be synapse-specific, induced immediately and last approximately an hour [12,13]. These changes might be implemented by modification of the NMDA receptor itself, or of some other post-synaptic protein, but are unlikely to involve changes in gene expression. Surprisingly, whether CaMKII has a role in such a modification has not yet been tested.

Another type of metaplasticity, of the sort envisioned by Bienenstock *et al.* [9], would not be strictly synapse-specific, in contrast to the first type. According to their theory, the threshold θ_m should respond to mean activity across the population of synapses, and in turn cause global shifts, if it is to be of advantage in achieving optimal stability and input selectivity. Because this kind of metaplasticity would act more generally, possibly over a slower time scale, changes in gene expression could be involved.

At this stage it is unclear whether the metaplasticity studied by Mayford *et al.* [1] belongs squarely in either of these two categories. In principle, expression of the CaMKII(T286D) transgene could mimic a global, nucleus-dependent change consistent with the latter type of metaplasticity. New gene expression is known to be required for the consolidation of long-term memory [26], as well as for the stabilization of long-lasting synaptic plasticity [27]; interestingly, expression of the endogenous gene for αCaMKII can be induced by strong synaptic stimulation in hippocampal neurons [28,29]. Might the arrival of new CaMKII molecules at a synapse support or trigger plasticity? Might it cause metaplasticity, or some other kind of change? Is it possible for the production and/or targeting of new CaMKII molecules to be synapse-specific, as plasticity and metaplasticity can be? Answers to these fundamental questions are likely to require new techniques, perhaps involving imaging and electrophysiology at single synapses.

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