distinctiveness of these two compartments. The memory $CD8^+$ population was clearly the less fastidious. Perhaps most striking was its ability to expand in the absence of specific antigen and to proliferate to a significant extent even in the absence of all MHC class I molecules. This observation recalls the recent finding that interferon-α can stimulate the proliferation of memory but not naïve $CD8^+$ cells in the absence of intentional antigenic stimulation (*5*). It is now imperative to determine how many such factors help to maintain the memory pool; in what contexts they are produced; how memory—but not naïve—cells stay attuned to them; and how they influence the mobilization of an antigen-specific memory response.

The fact that naïve $CD8^+$ T cells require contact with the correct MHC class I molecule in order to survive for prolonged periods in the periphery hints at a process akin to positive selection in the thymus. Positive selection favors export of a useful T cell repertoire by promoting the survival and differentiation of only those thymocytes that can productively interact with the MHC molecules expressed on thymic stromal cells. That such a peripheral selection process might be generally occurring is supported by two recent reports, relying on nontransgenic systems, that $CD4^+$ T cells survive for much longer periods in the periphery if they make contact with MHC class II molecules (*6*, *7*). Some of the questions immediately raised are whether the repertoire of T cell specificities undergoes any significant further shaping as a result of peripheral selection, which peripheral cells need to express MHC molecules in order to enhance survival, and precisely what receptor interactions and internal signals are involved.

These new results preview a story that will demand our close attention as it unfolds.

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NEUROSCIENCE

Learning Mechanisms: The Case for CaM-KII

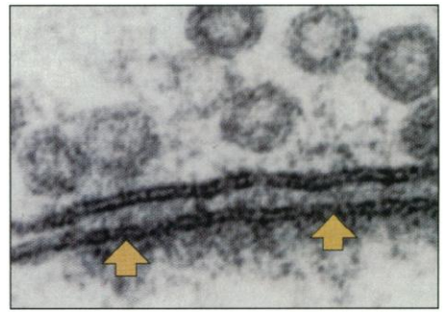
John Lisman, Robert C. Malenka, Roger A. Nicoll, Roberto Malinow

What are the long-lasting changes that occur in your brain as you learn new information? It is generally thought that memory is due to persistent modification of the strength of synapses, the structures that communicate information from one neuron to the next. One such modification is the long-term potentiation (LTP) that occurs at the synapses of the CA1 region of the hippocampus. This type of modification is of particular interest because it has associative properties that match that of learning itself (we come to associate the smell and taste of food), and because the hippocampus is important for long-term memory. The report by Barria *et al.* on page 2022 in this issue (*1*) is a significant step forward in our understanding of the persistent biochemical modifications that underlie this form of LTP.

The major new finding of Barria *et al.* is that the postsynaptic receptors (a subtype of glutamate receptor known as AMPA receptors) that mediate excitatory synaptic transmission at this synapse become phosphorylated after LTP induction and stay phosphorylated for at least 1 hour thereafter. The authors also show that phosphorylation of AMPA receptors in an expression system enhances the responses of these receptors to glutamate. Together with previous findings (*2*), these results provide strong evidence for a simple, postsynaptic mechanism for enhancing synaptic transmission during LTP.

The initial triggering event of LTP, believed to be a brief rise in postsynaptic calcium, results in the phosphorylation of AMPA receptors. What causes this modification and how is it maintained for at least an hour after the initial triggering event? A large body of evidence suggests that calmodulin-dependent protein kinase II (CaM-KII) is a critical player in LTP, and it has special properties that make it an attractive candidate for exhibiting persistent changes and serving as a memory molecule (*3*). CaM-KII is localized in the postsynaptic density, directly adjacent to the channels that mediate synaptic transmission (see the figure). The work of Barria *et al.* suggests that CaM-KII controls AMPA receptors directly, because the phosphorylation of AMPA receptors after LTP induction occurs at a site that can be phosphorylated by CaM-KII and is blocked by an inhibitor of CaM-KII. Theoretical (*4*) and experimental (*5*) studies suggest that the maintenance of the AMPA receptor phosphorylation may be due to the ability of CaM-KII to maintain its activity for long periods after its initial activation by calcium. The kinase may accomplish this by calcium-dependent autophosphorylation of the threonine residue at position 286, which renders its activity independent of calcium. This autocatalytic process could maintain the "on" state of the kinase for long periods, perhaps indefinitely. Indeed, Barria *et al.* provide support for this mechanism by showing that CaM-KII stays persistently phosphorylated at the 286 site for at least 1 hour after LTP induction [but see (*6*)]. Other kinases may also contribute to this persistent phosphorylation (*7*), as inhibitors of CaM-KII have failed to depress established LTP (*8*).

A simple and direct role for CaM-KII in triggering and perhaps maintaining LTP is supported by studies in which CaM-KII activity was acutely increased either with viral transfection (*9*), injection of the active enzyme (*10*), or injection of calcium and calmodulin (*11*). In these cases synaptic transmission is enhanced and LTP is occluded. However, recent work (*12*) with transgenic mice, in which constitutively ac-



Where LTP happens. The postsynaptic side of the synapse (arrows) is the site of persistent biochemical modifications that maintain LTP. [Photo courtesy of Kristen Harris]

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tive CaM-KII was chronically expressed, appears to contradict this view of the role of CaM-KII in LTP. Such mice exhibit no increase in synaptic transmission, and normal LTP can still be generated (although there is a shift in the frequency dependence of LTP). These results led to the conclusion that CaM-KII is not part of the direct signal transduction cascade responsible for generating LTP but only modulates this machinery.

Can these conflicting data be reconciled? One possibility is that CaM-KII does directly mediate the generation of LTP but can also modulate the sensitivity of this transduction pathway. In particular, chronic CaM-KII activity (*12*) could decrease the sensitivity of this pathway (perhaps by phosphatase activation). This view is a special case of the "sliding threshold" model (*13*) in which a requisite component of the signal transduction pathway used to generate LTP (that is, CaM-KII) can itself also act to modulate the pathway. This modulation may occur primarily as a result of long-term genetic modifications and not of more acute perturbations. A homeostatic compensatory regulation of this kind may be an example of a general principle in biology that serves to establish a functionally relevant dynamic range for signal transduction. Simply put, if a pathway is continually activated it will tend to decrease its sensitivity; if it is not activated it will increase its sensitivity.

Clear examples of how difficult it is to interpret the results of genetic modification of transduction cascades comes from work on rod phototransduction (*14*). The advantage of this system is that the molecular basis of the cascade is known, so one can assess whether genetic modifications produce easily interpretable effects. Transgenic mice expressing a constitutively active transducin that mediates phototransduction would be expected to show saturation of the subsequent transduction cascade. However, a compensatory reduction in downstream enzymes prevents saturation. Another example comes from mice in which the inhibitory subunit of cyclic guanosine 3′,5′-monophosphate (cGMP)–phosphodiesterase is deleted. This would be expected to lead to a drop in cGMP concentration. Surprisingly, cGMP concentration is elevated as a result of an unanticipated disappearance of the catalytic subunits of the enzyme. In many other cases, modification of rod proteins leads to cell-specific degeneration. These examples indicate that extreme caution must be used in interpreting results obtained by genetic modification of signal transduction pathways, especially when genetic and anatomical controls are not feasible.

In theory, compensatory changes might be minimized by inducible genetic modification. For instance, heat shock promoters have been used in *Drosophila* to produce changes in gene expression within 30 min. The elegant new methods (*15*) for inducible gene expression in mice are comparatively slow (2 weeks) and may not eliminate the problems of compensatory change.

With the new results from Barria *et al.*, a strong case is emerging for the importance of CaM-KII in the direct control of synaptic strength and, by implication, in the storage of information (*16*). CaM-KII, as part of an important synaptic signal transduction pathway, may also control homeostatic pathways, which only become apparent during chronic manipulations, such as those that occur with current methods for genetic manipulations.

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IMAGING

Tissue Optics

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Our bodies are not impervious to light. When you step outside on a sunny day, the inside of your head is illuminated—in fact, sufficient light penetrates your skull to comfortably read this page (*1*). Shine a flashlight at your finger, and your finger will glow red, a demonstration that red and near-infrared light penetrate deeply into tissue. Just as farmers once candled eggs to check for chick embryos, transillumination has been used to screen for scrotal tumors, breast cancer, and blood oxygenation (*2*). Although the resolution and diagnostic accuracy of these early attempts at imaging with light were poor, this is changing. In this issue on page 2037, Tearney *et al.* (*3*) use light-based optical coherence tomography (OCT) to form tomographic images of human tissue in situ with a resolution exceeding that of magnetic resonance imaging (MRI), computerized tomography (CT), or ultrasound.

In tissue, light is both absorbed and scattered; scattering dominates in the red and near-infrared spectrum (see the figure). This native scattering renders us opaque and blurs transmission images. However, those photons surviving passage through the tissue emerge bearing clues about their voyage. Study of their behavior has spawned a field now known as tissue (or biomedical) optics (see related News story on page 1991). Optical approaches to tissue imaging have general advantages: They are inexpensive, noninvasive, transportable, and nontoxic. In addition, biological materials have native contrast that allows optical assessment of tissue chemistry and cellular structure.

OCT takes advantage of the scattering inherent in tissue to generate in vivo images. As photons meander through tissue, a small amount of light that has bounced off internal structures only once is coherently back-reflected from various boundaries within the tissue. Using interferometric techniques, this coherently backscattered light can be detected at intensities down to 1 part in 10^{11}, while simultaneously revealing the depth of the scattering event. At the heart of OCT is a Michelson interferometer, which detects interference between halves of a single light beam split along a sample arm, containing low-coherence light backscattered from multiple depths within the tissue; and a reference arm, containing light passing through an air or water gap of variable length (*4*). When sample and reference light are recombined, and the time delay through both paths matches within the coherence

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