

# A fresh look at the role of CaMKII in hippocampal synaptic plasticity and memory

Christopher Rongo

## Summary

Advances in molecular, genetic, and cell biological techniques have allowed neuroscientists to delve into the cellular machinery of learning and memory. The calcium and calmodulin-dependent kinase type II (CaMKII) is one of the best candidates for being a molecular component of the learning and memory machinery in the mammalian brain. It is present in abundance at synapses and its enzymatic properties and responsiveness to intracellular  $\text{Ca}^{2+}$  fit a model whereby  $\text{Ca}^{2+}$  currents activate the kinase and lead to changes in synaptic efficacy. Indeed, such plastic properties of synapses are thought to be important for memory formation. Genetic analysis of the alpha isoform of CaMKII in mice support the hypothesis that CaMKII signaling is required to initiate the formation of new spatial memories in the hippocampus. CaMKII is also required for the correct induction of long-term potentiation (LTP) in the hippocampus, consistent with the widely held belief that LTP is a mechanism for learning and memory. Recent cell biological, genetic, and physiological analyses suggest that one of the cellular explanations for LTP and CaMKII function might be the trafficking of AMPA-type receptors to synapses in response to neural activity. *BioEssays* 24:223–233, 2002. © 2002 Wiley Periodicals, Inc.; DOI 10.1002/bies.10057

## Introduction

A major goal in neuroscience is to determine how the nervous system acquires and stores memories. For many years neuroscientists were limited to anatomical explanations for the mechanisms of learning and memory based on psychological examinations of patients with lesions in specific brain

regions. Human patients and experimental animals with hippocampal lesions in particular have been informative in the deconstruction of memory. First, hippocampal lesions showed that two types of memory, implicit and explicit, which were psychologically defined, could now be distinguished anatomically as well. Implicit memory typically involves unconscious recall such as in training reflexive motor skills, whereas explicit memory involves factual information that is recalled through conscious effort. Patients with hippocampal lesions often had deficits in explicit memory whereas their implicit memory remained intact. Second, hippocampal lesions revealed that memory formation is a step-wise process in which memories pass through stages over time, and these stages could be defined anatomically. For example, patients with hippocampal lesions can recall old memories (long-term memory) but are unable to store new memories, suggesting that the hippocampus acts as a way-station that transforms short-term memories into long-term ones, perhaps by transferring the acquired information and the relevant contextual associations to the cortex.

With the advent of modern molecular and genetic techniques during the last thirty years, we have been faced with the challenge of moving from an anatomical definition of memory to a molecular one. As neurons are the basic functional unit of the nervous system, much emphasis has been placed on understanding how neurons communicate at the cellular and molecular level. Evidence is building for a relationship between hippocampal long-term potentiation (LTP) as a mechanism for modulating neuron–neuron communication, and hippocampus-dependent memory. In this review, I discuss how the tools of genetics, cell biology, and electrophysiology have helped neuroscientists elucidate the mechanisms underlying learning and memory in the hippocampus. In particular, I focus on CaMKII and its role as a key regulator in these molecular mechanisms.

## LTP as a molecular model for memory

First to consider the problem of memory acquisition at the level of individual cells. The simplest cellular model for learning and memory over the last fifty years has been Hebb's rule concerning synaptic plasticity, whereby coincident activity in two connected neurons results in the strengthening of their connection.<sup>(1)</sup> In the simplest example, one neuron, termed

Funding agency: Pew Scholars in the Biomedical Sciences.  
Waksman Institute/Rutgers University, 190 Frelinghuysen Rd,  
Piscataway, NJ.  
E-mail: rongo@waksman.rutgers.edu

Abbreviations: AMPA,  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; CaMKII, calcium and calmodulin-dependent kinase type II; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; LTD, long-term depression; LTP, long-term potentiation; mEPSP, miniature excitatory postsynaptic potential; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PTP, post-tetanic potentiation; STP, short-term potentiation.

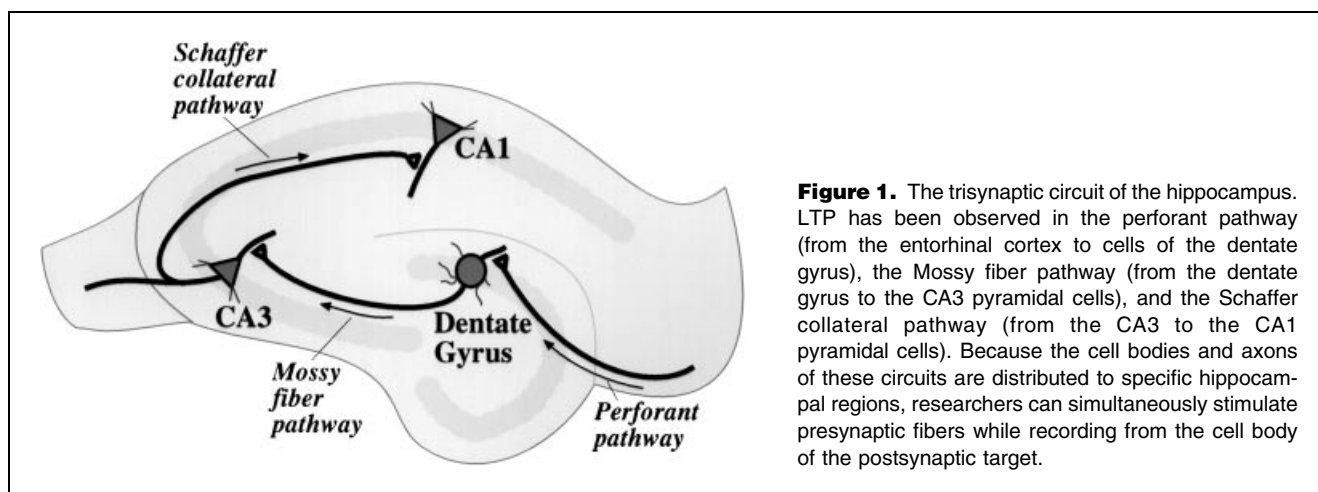
the presynaptic neuron, synapses on to a second neuron, termed the postsynaptic neuron. Under normal circumstances, activity in the presynaptic neuron rarely causes the postsynaptic neuron to fire; the efficacy of communication between the neurons is low. However, if a presynaptic neuron is able to excite a postsynaptic neuron by repeatedly causing that neuron to fire such that both neurons eventually become active at the same time, then physiological changes are induced that increase the ability of the presynaptic neuron to fire the postsynaptic neuron. Low-level neural activity at such a circuit might be ignored prior to such an increase in efficacy; however, after an increase in efficacy, low-level neural activity would result in a large postsynaptic response at that specific circuit. In a probably oversimplified interpretation of this model, new memories might be brought about through specific circuits of connected neurons becoming stronger as the neurons are repeatedly used in a coincident fashion.

One physiological phenomenon that fits Hebb's rule is long-term potentiation (LTP), first discovered in the hippocampus by Bliss and Lomo.<sup>(2)</sup> LTP is defined as a long-lasting increase in synaptic efficacy between two neurons that results after a brief high-frequency stimulation, and it has been widely studied as a promising cellular mechanism for learning and memory. LTP has been observed in the three major interconnected synaptic circuits of the hippocampus: the perforant pathway from the entorhinal cortex to dentate gyrus, the Mossy fiber pathway from dentate gyrus to the CA3 pyramidal cells, and the Schaffer collateral pathway from the CA3 to the CA1 pyramidal cells<sup>(2-4)</sup> (Fig. 1). LTP can be monitored in individual pathways because the regional anatomy of the hippocampus allows the stimulation of specific afferent axons in one region of the hippocampus while simultaneously recording from a population of the cell bodies of their postsynaptic targets in another region of the hippocampus. Prior to an LTP-inducing stimulation, a baseline excitatory postsynaptic poten-

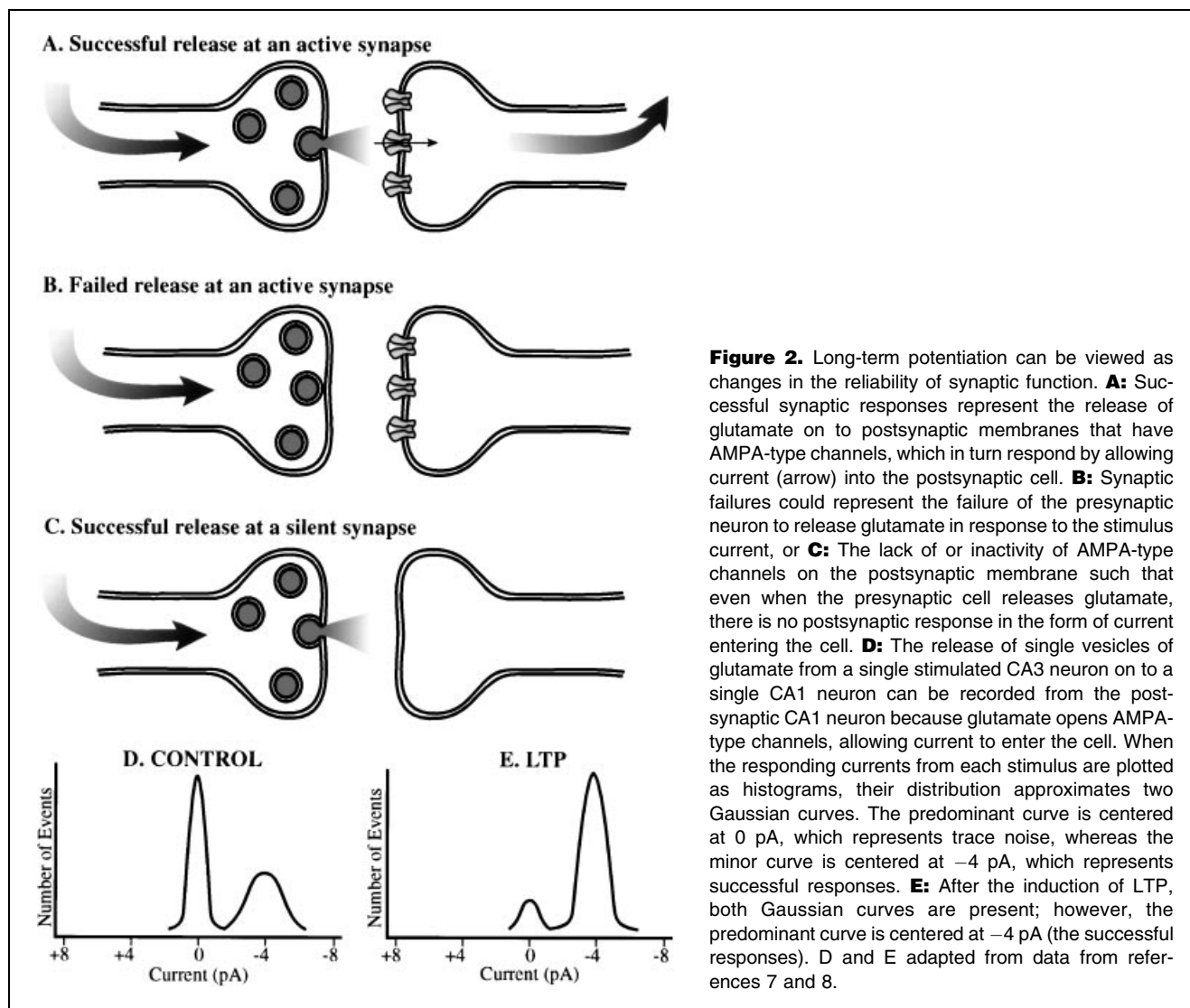
tial (EPSP) slope can be recorded from the postsynaptic cells, and this variable is a useful measure of the typical strength of a postsynaptic response. After a brief high frequency stimulus is applied, LTP is measured as the percentage increase in the EPSP slope relative to the baseline EPSP slope recorded prior to LTP induction. LTP fits Hebb's rule because it shows that a specific circuit can increase its synaptic efficacy in response to high frequency excitation and coincident firing between presynaptic and postsynaptic cells.

Potentiation in the hippocampus can be further defined by its temporal properties.<sup>(5,6)</sup> After an LTP-inducing stimulation, the initial synaptic potentiation decays within minutes, and is called post-tetanic potentiation (PTP). Under certain conditions, one can observe short-term potentiation (STP), which usually lasts about 15 minutes. LTP can be defined by its early or late phases, where early LTP lasts hours and does not depend on new protein synthesis, and late LTP can last days and requires new protein synthesis. Hippocampal LTP also has unique physiological properties depending on which synaptic circuit is being described.<sup>(5,6)</sup> Thus, multiple molecular mechanisms might lead to the electrophysiological phenomena of LTP, and these different mechanisms might be utilized at different circuits.

How exactly is synaptic efficacy increased during LTP? There are several components to consider as possible targets of regulation. For example, the presynaptic cell encapsulates neurotransmitter within synaptic vesicles, which fuse with the synaptic membrane to release their neurotransmitter contents when the presynaptic neuron fires. Neurotransmitter binds to receptors on the postsynaptic membrane, causing them to open so that ionic current enters and depolarizes the cell (Fig. 2A). One possible mechanism for increasing efficacy would be to load a vesicle with more transmitter. Alternatively, efficacy could be increased by increasing the probability that any one vesicle will fuse in response to the firing of the



**Figure 1.** The trisynaptic circuit of the hippocampus. LTP has been observed in the perforant pathway (from the entorhinal cortex to cells of the dentate gyrus), the Mossy fiber pathway (from the dentate gyrus to the CA3 pyramidal cells), and the Schaffer collateral pathway (from the CA3 to the CA1 pyramidal cells). Because the cell bodies and axons of these circuits are distributed to specific hippocampal regions, researchers can simultaneously stimulate presynaptic fibers while recording from the cell body of the postsynaptic target.



**Figure 2.** Long-term potentiation can be viewed as changes in the reliability of synaptic function. **A:** Successful synaptic responses represent the release of glutamate on to postsynaptic membranes that have AMPA-type channels, which in turn respond by allowing current (arrow) into the postsynaptic cell. **B:** Synaptic failures could represent the failure of the presynaptic neuron to release glutamate in response to the stimulus current, or **C:** The lack of or inactivity of AMPA-type channels on the postsynaptic membrane such that even when the presynaptic cell releases glutamate, there is no postsynaptic response in the form of current entering the cell. **D:** The release of single vesicles of glutamate from a single stimulated CA3 neuron on to a single CA1 neuron can be recorded from the postsynaptic CA1 neuron because glutamate opens AMPA-type channels, allowing current to enter the cell. When the responding currents from each stimulus are plotted as histograms, their distribution approximates two Gaussian curves. The predominant curve is centered at 0 pA, which represents trace noise, whereas the minor curve is centered at -4 pA, which represents successful responses. **E:** After the induction of LTP, both Gaussian curves are present; however, the predominant curve is centered at -4 pA (the successful responses). D and E adapted from data from references 7 and 8.

presynaptic neuron. This model assumes that prior to LTP induction a firing of a particular presynaptic neuron does not always result in a vesicle fusion and neurotransmitter release (Fig. 2B). On the postsynaptic side of the synapse, the neurotransmitter receptor could be regulated such that the probability of the postsynaptic neuron to respond to neurotransmitter is increased. This model assumes that prior to LTP induction a particular postsynaptic neuron does not always respond to neurotransmitter released by the presynaptic cell (Fig. 2C). Alternatively, the magnitude of the postsynaptic response itself could be increased in response to LTP induction. Any of these mechanisms, or some combination of them, could theoretically explain LTP.

One breakthrough for addressing these possibilities was the examination of LTP from recordings of a single presynaptic CA3 neuron exciting a single postsynaptic CA1 neuron in the

Schaffer collateral pathway.<sup>(7,8)</sup> A single stimulation of a CA3 neuron at a low rate can, but does not always, result in a postsynaptic response in the CA1 neuron (Fig. 2D). The resulting electrophysiological traces, when plotted as amplitude histograms, can be described by two Gaussian distributions: one corresponding to the noise in the traces and one that is similar in amplitude to miniature synaptic potentials (mEPSPs) from spontaneous release of single vesicles. The results of many of these experiments suggest that a pair of connected CA3 and CA1 neurons might have only one functional synapse, at which only a single vesicle is released in an all or nothing (i.e., quantal) manner in response to the firing of the presynaptic CA3 neuron. After LTP is induced in this circuit, a single synapse still releases one synaptic vesicle when stimulated at a low rate, and the postsynaptic response to the release of one vesicle is thought to remain the same in

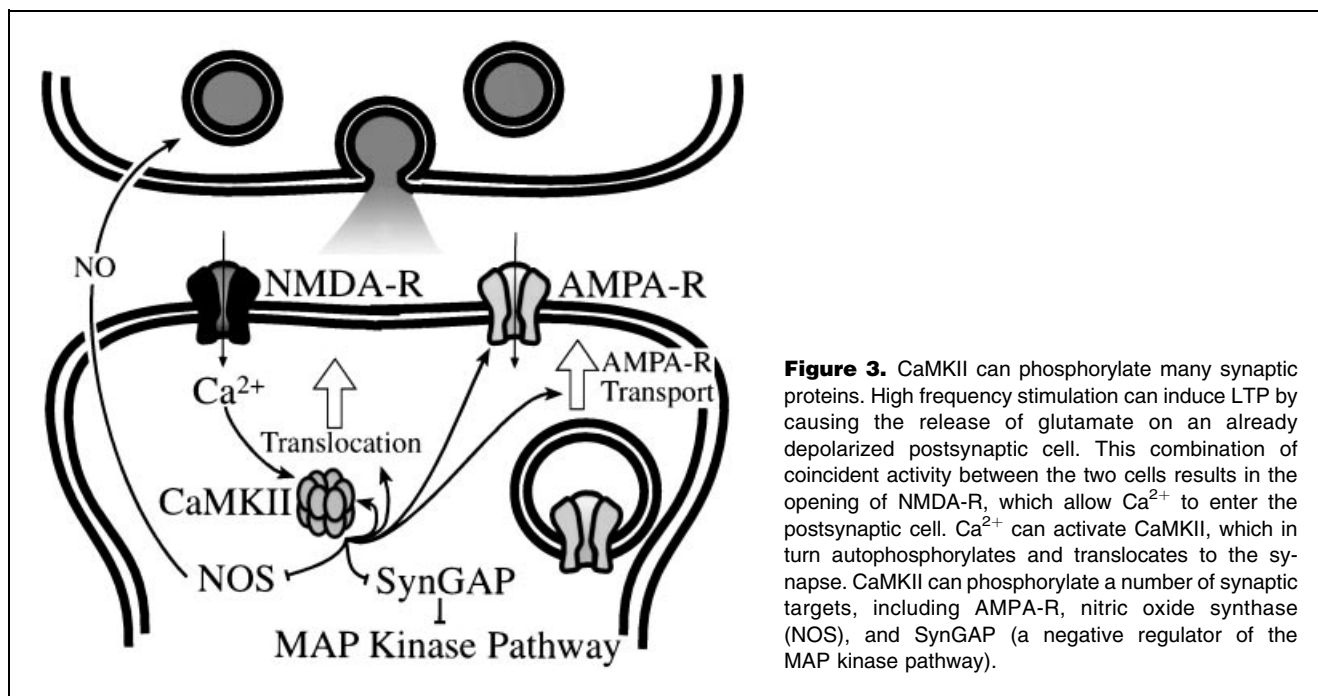
most cases. The primary observed change is that the probability of a response has increased (Fig. 2E). The easiest explanation for these results at the time that the experiments were conducted was that an action potential in the presynaptic CA3 neuron still releases synaptic vesicles after LTP induction, but does so with a higher probability of success.

### LTP is initiated in the postsynaptic cell (CA1) and requires NMDA receptors

Quantal physiological experiments suggested a model in which LTP causes an increase in the synaptic vesicle release probability. In order to fit Hebb's rule, the presynaptic and postsynaptic neurons in this model must be excited coincidentally for synaptic efficacy to be increased. How is coincidence measured, and what is the role of the postsynaptic cell in LTP? Presumably there must be a molecule that acts as the coincidence detector. The first clues to these questions came from studies of the glutamate receptors. CA1 neurons express at least two types of these receptors: NMDA (N-methyl-D-aspartate) and AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionate). AMPA-type receptors (AMPA-R) form ligand-gated ion channels that open in response to glutamate to allow  $\text{Na}^+$  and  $\text{K}^+$  to move through the channel and depolarize the cell. NMDA-type receptors (NMDA-R) also form ligand-gated ion channels; however, these channels only open in response to glutamate when the postsynaptic membrane is depolarized, thereby relieving a  $\text{Mg}^{2+}$  block.<sup>(9,10)</sup> This property of the NMDA-R makes them good candidates for coincidence detectors that monitor the coincident firing of the presynaptic and postsynaptic cells: the receptors only open when they

simultaneously detect glutamate released from the active presynaptic cell and depolarization of the active postsynaptic cell. Coincident firing of presynaptic and postsynaptic cells would result in the release of glutamate into the synaptic cleft by the presynaptic cell at the same time that the postsynaptic cell is depolarized, the two conditions needed to cause NMDA-R to open. Indeed, LTP is inhibited in hippocampal CA1 recordings to which pharmacological agents that specifically blocked NMDA-R from opening have been added, suggesting that NMDA-R-signaling in the postsynaptic cell is crucial for the formation of LTP.<sup>(11)</sup>

Next to consider how NMDA-R contribute to LTP and what makes the signaling by these receptors unique and important relative to other glutamate-gated channels. Is it possible that the NMDA contribution to synaptic current is important for general synaptic function, and therefore LTP? One interesting property of NMDA-R is that they allow not only  $\text{Na}^+$  and  $\text{K}^+$  to pass through, but they also allow  $\text{Ca}^{2+}$  to enter the postsynaptic cell, suggesting that  $\text{Ca}^{2+}$  is the critical signal for coincident activity.<sup>(12-14)</sup> The addition of  $\text{Ca}^{2+}$  chelators to the postsynaptic cell prevents the formation of LTP, consistent with the idea that  $\text{Ca}^{2+}$  entry through NMDA-R induces LTP.<sup>(15,16)</sup>  $\text{Ca}^{2+}$  can activate several kinase signaling molecules, including calcium and calmodulin-dependent kinase II (CaMKII), protein kinase C (PKC), and the fyn tyrosine kinase.<sup>(17-21)</sup> One interesting model suggested by these studies is that  $\text{Ca}^{2+}$  influx from NMDA-R in turn activates  $\text{Ca}^{2+}$ -inducible kinases like CaMKII, which in turn phosphorylate synaptic proteins and thereby facilitate the increase in synaptic efficacy that results during LTP (Fig. 3).



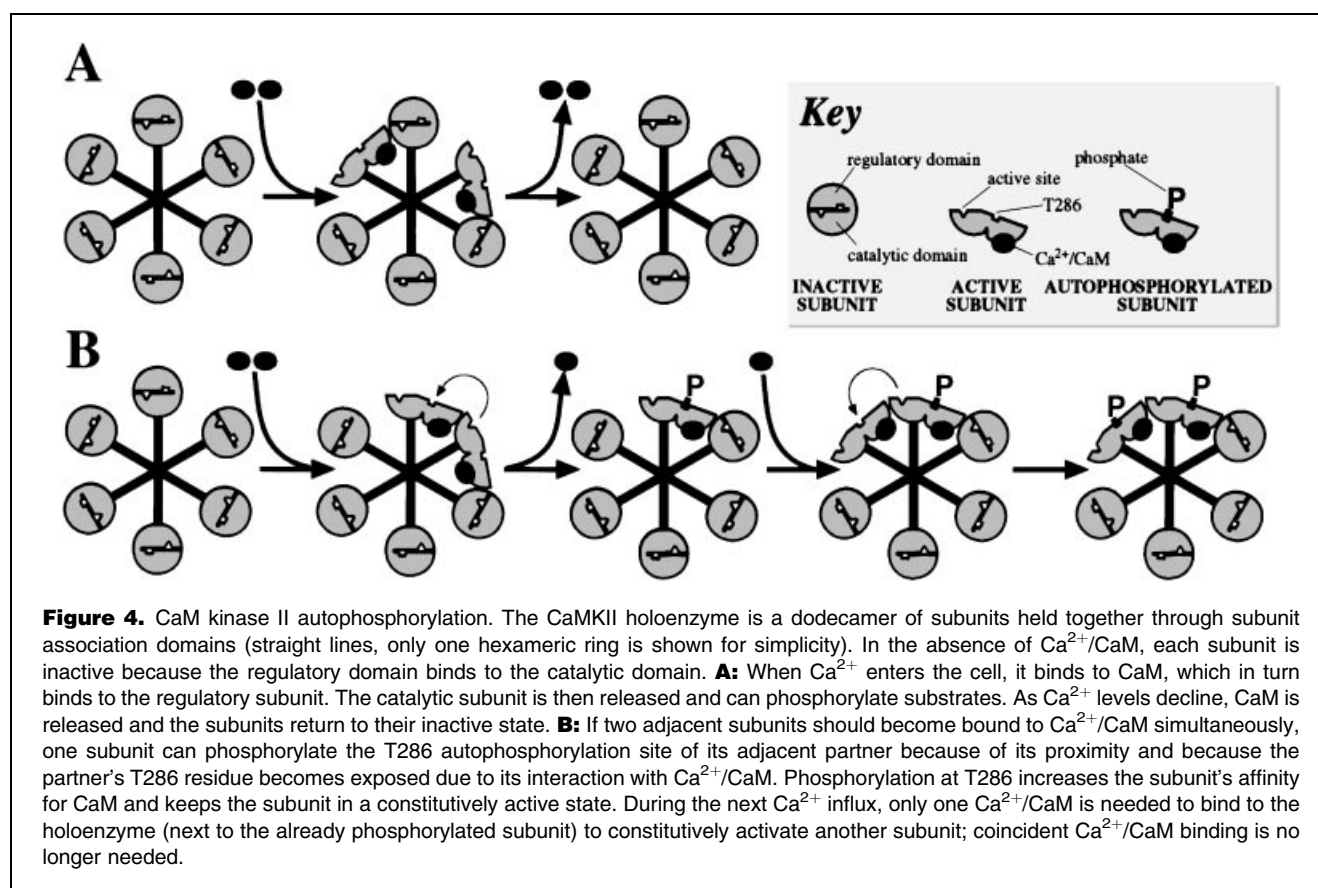
**Figure 3.** CaMKII can phosphorylate many synaptic proteins. High frequency stimulation can induce LTP by causing the release of glutamate on an already depolarized postsynaptic cell. This combination of coincident activity between the two cells results in the opening of NMDA-R, which allow  $\text{Ca}^{2+}$  to enter the postsynaptic cell.  $\text{Ca}^{2+}$  can activate CaMKII, which in turn autophosphorylates and translocates to the synapse. CaMKII can phosphorylate a number of synaptic targets, including AMPA-R, nitric oxide synthase (NOS), and SynGAP (a negative regulator of the MAP kinase pathway).

### CaMKII as a sensor of $\text{Ca}^{2+}$ oscillations

CaMKII was originally identified as an abundant brain protein found in biochemical fractions that were enriched for post-synaptic density proteins.<sup>(22–25)</sup> In mammals, CaMKII is encoded by four alternatively spliced genes ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), with  $\alpha$  and  $\beta$  being the predominantly expressed isoforms in neurons. Each isoform comprises several motifs, including an N-terminal catalytic domain, an autoinhibitory domain, a  $\text{Ca}^{2+}/\text{CaM}$ -binding domain, and a C-terminal subunit association domain. Individual subunits join together through subunit association domains to form a holoenzyme, which is thought to be arranged in two stacks of hexameric rings.<sup>(26)</sup> In the absence of  $\text{Ca}^{2+}/\text{CaM}$ , CaMKII is thought to be inactive because the autoinhibitory domain binds to the catalytic domain, thereby preventing access to substrate (Fig. 4). In the presence of  $\text{Ca}^{2+}/\text{CaM}$ , the  $\text{Ca}^{2+}/\text{CaM}$ -binding domain interacts with  $\text{Ca}^{2+}/\text{CaM}$ , and this interaction is thought to disrupt the interaction of the autoinhibitory domain with the catalytic domain. Once freed from binding the autoinhibitory domain, the catalytic domain can now phosphorylate substrate molecules.<sup>(27–29)</sup> There is a threonine (T286) in the CaMKII autoinhibitory domain that fits the CaMKII phosphorylation consensus site.<sup>(30,31)</sup> Thus, if two adjacent subunits in the

holoenzyme both bind  $\text{Ca}^{2+}/\text{CaM}$ , one subunit will expose its T286 residue, whereas the adjacent subunit will expose and activate its catalytic domain so that it can phosphorylate the adjacent T286.<sup>(32,33)</sup> This intersubunit autophosphorylation will prevent the autoinhibitory domain from binding and inhibiting the catalytic domain, regardless of the presence of  $\text{Ca}^{2+}/\text{CaM}$ .<sup>(28–30,34)</sup> Autophosphorylated CaMKII retains partial activity, and is thus  $\text{Ca}^{2+}/\text{CaM}$ -independent and constitutively active.

The autoregulatory properties of CaMKII subunits within the holoenzyme make CaMKII an excellent sensor of intracellular  $\text{Ca}^{2+}$  concentration and oscillations.<sup>(35)</sup> Autophosphorylation requires coincident binding of two  $\text{Ca}^{2+}/\text{CaM}$  molecules to two adjacent subunits. With low frequency oscillations,  $\text{Ca}^{2+}/\text{CaM}$  is not present for a period of time long enough to ensure that two molecules of  $\text{Ca}^{2+}/\text{CaM}$  can bind to two adjacent subunits (Fig. 4A). As the frequency of oscillations increases, this probability of coincident binding increases until autophosphorylation results in one active subunit within the holoenzyme (Fig. 4B). Because one subunit is now active and  $\text{Ca}^{2+}/\text{CaM}$ -independent, coincident binding is no longer needed to get additional subunits phosphorylated. If one molecule of  $\text{Ca}^{2+}/\text{CaM}$  binds to a subunit that is adjacent to the



already activated subunit within the holoenzyme, the newly bound subunit will be immediately phosphorylated, creating a holoenzyme with two active subunits. Thus, critical frequencies of intracellular  $\text{Ca}^{2+}$  oscillation will result in distinct levels of holoenzyme kinase activity due to autophosphorylation cooperativity.

### CaMKII is required for LTP and spatial learning

Because CaMKII autophosphorylation could result in  $\text{Ca}^{2+}$ /CaM-independent kinase activity long after the initial  $\text{Ca}^{2+}$  signaling, it was suggested that CaMKII autophosphorylation might represent a type of molecular memory.<sup>(30,36)</sup> Indeed, pharmacological agents that inhibit CaMKII, calmodulin, or general kinase inhibitors have been shown to block LTP when added to recordings from hippocampal slices.<sup>(18,19,37,38)</sup> To determine if CaMKII played an important role in learning, memory and LTP, Silva et al. set out to knock out the alpha isoform of CaMKII in mice through homologous recombination.<sup>(39)</sup> Mice that lack  $\alpha$ -CaMKII are viable, fertile, and generally healthy. Hippocampal NMDA and AMPA receptor synaptic currents are intact, and PTP can be observed in response to high-frequency stimulation. Hippocampal STP and LTP are reduced, however, in these mice.<sup>(39,40)</sup>

Previous evidence supporting the notion that LTP was the underlying mechanism for learning and memory was based on the application of NMDA receptor channel antagonists that prevent LTP and impair learning and memory in rats.<sup>(41,42)</sup> However, because the NMDA-R contribute to the synaptic current, it is easy to imagine how their impairment by channel antagonists could block learning and memory simply by altering synaptic function, and not by blocking LTP. In contrast,  $\alpha$ -CaMKII knockout mice are not impaired for synaptic function, but are deficient in LTP; thus, tests for learning and memory deficits in these mice could be more readily interpreted.<sup>(43)</sup> Lesions in the hippocampus are known to cause deficits in spatial memory, and such deficits are revealed in rodents through tests such as the Morris water maze in which mice must navigate their way through a pool of opaque water by learning visual clues. Mice lacking  $\alpha$ -CaMKII are impaired for spatial memory tasks like the Morris water maze; in contrast, they can perform well at tasks that require non-spatial associative memory. Whereas these studies of the  $\alpha$ -CaMKII knockout mice supported the notion that LTP was a mechanism for spatial learning and memory, they could not rule out a role for CaMKII during the development of the brain that would be required later in life to acquire and store memories.

The role of CaMKII autophosphorylation was also examined using transgenic mice. Autophosphorylation of CaMKII can be mimicked by a mutation of threonine 286 to an aspartate (CaMKII(T286D)), resulting in a constitutively active kinase that does not require an initial  $\text{Ca}^{2+}$  stimulus to become active.<sup>(28–30,34)</sup> When a transgene that expresses CaM-

KII(T286D) was introduced into the mouse germline, the resulting mice exhibit normal CA1 LTP in response to high-frequency stimulation.<sup>(44,45)</sup> However, stimulation at lower frequencies that normally can induce LTP in wild-type mice instead induces long-term depression (LTD) in CaMKII(T286D) transgenic mice such that their EPSP slope becomes smaller relative to baseline after stimulation. One elegant aspect to this experiment is that the transgene is under the control of a forebrain-specific promoter combined with the tetracycline transactivator system. Thus, expression of the transgene is tissue specific and under temporal control. Mayford and colleagues could induce the expression of the transgene long after development and still detect an impairment both in LTP formation in response to low-frequency tetanic stimuli and in the ability of the mice to perform spatial learning and memory tests. Moreover, they could give the mice doxycycline to turn the transgene off, then retest the mice for LTP or learning and memory. Their results showed that CaMKII(T286D) transgenic mice have LTP deficits as well as learning and memory deficits that are reversible upon addition of doxycycline. This strongly argued that mice expressing CaMKII(T286D) have acute effects in LTP and memory formation, rather than irreversible defects in brain development that could subsequently impair the ability of mice to learn spatial tasks. When one compares the phenotypes of  $\alpha$ -CaMKII knock-out mice and CaMKII(T286D) transgenic mice, however, we are left with the puzzling conclusion that too little or too much CaMKII activity impairs LTP and learning.

This topic also brings us to the question of how CaMKII could be facilitating LTP (Fig. 3). Autophosphorylation of CaMKII results in its translocation to the synapse, which could prolong its response to  $\text{Ca}^{2+}$  influx.<sup>(46)</sup> CaMKII has been shown to phosphorylate a number of postsynaptic proteins, including the AMPA-R and signal transduction molecules like SynGAP, which inhibits the MAP kinase pathway.<sup>(47–50)</sup> The phosphorylation of such postsynaptic substrates could be responsible for the physiological changes in the postsynaptic cell needed for LTP.

Could CaMKII activation in the postsynaptic cell also result in physiological changes in the presynaptic cell? Because one can interpret LTP as a change in the probability of vesicle release in the presynaptic cell, it has been postulated that NMDA receptors and CaMKII signaling in the postsynaptic cell result in the production of a retrograde messenger that can signal back to the presynaptic cell (Fig. 3). One candidate retrograde messenger is nitric oxide (NO). NO is produced by nitric oxide synthase (NOS), and double mutant mice lacking both neuronal and endothelial forms of NOS show impaired CA1 LTP.<sup>(51)</sup> It has been shown that CaMKII can phosphorylate NOS, resulting in decreased NO production.<sup>(52–54)</sup> This observation would suggest a model whereby  $\text{Ca}^{2+}$  signaling in the postsynaptic cell results in phosphorylation of NOS by CaMKII and subsequent reduction of the NO levels, although

how decreased NO would act as a retrograde messenger to induce LTP in the presynaptic cell is unclear.

### Silent synapses as a model for LTP

Several problems with the presynaptic model of LTP have come up over the years as researchers have probed what is probably the most controversial aspect of LTP research (see reviews, Refs. 55–57). First, physiological manipulations such as LTP that increase the probability of neurotransmitter release should cause an equal increase in NMDA-R and AMPA-R synaptic response; however, often AMPA-R response is increased to a greater extent than NMDA-R response. Second, more direct measurements of glutamate release show that LTP does little to increase such release. This remains controversial, however, since recent measurements of vesicle release observed using FM1-43 dye support a presynaptic role.<sup>(58)</sup>

In order to obtain a better understanding of LTP, a number of groups have taken a cell biological approach to studying CA1 LTP by focusing on the postsynaptic cell. Recall that LTP can be measured as an increase in the release probability of single synaptic vesicles from a presynaptic neuron stimulated to fire. These single vesicle events are measured by recording from the postsynaptic cell. It had been assumed that the failure of the postsynaptic cell to respond to a stimulated presynaptic cell indicates a failure of the presynaptic cell to release a single synaptic vesicle (Fig. 2B), and that LTP reflects an increase in the single vesicle release probability. An alternative explanation is that the single vesicle release probability does not change, and that instead the probability of whether the postsynaptic cell responds or not is increased by LTP. How could postsynaptic response have such a quantal, all or none character? One possibility is that the quanta are the synapses themselves and that, although a number of synapses might exist between two neurons, only a small fraction of them might be active (i.e., capable of responding to synaptic vesicle release from the presynaptic cell). The remaining synapses are termed “silent,” in that they do not contribute to EPSPs in response to synaptic vesicle release from the presynaptic cell (Fig. 2C). In this model, LTP increases synaptic efficacy by converting silent synapses into active synapses. Data that classically interpreted LTP as an increase in the chance that a synapse from a presynaptic neuron would release glutamate could now be explained as an increase in the number of synapses on the postsynaptic cell capable of responding to glutamate.

Do silent synapses exist? Such synapses can be loosely defined as synapses that possess the structural elements of synapses as detected by electron microscopy, but fail to produce a physiological response in the receiving cell (see review, Ref. 55). With respect to the hippocampal Schaffer collateral pathway where correlations between synaptic ultrastructure and synaptic physiology have been difficult,

silent synapses have been defined instead as synapses that signal only by NMDA-R, which due to their physiological properties do not produce a response at resting potentials. Several studies were able to show that developing neurons in the vertebrate CNS contained a significant number synapses with only NMDA-R (see review, Ref. 59). Upon maturation of the nervous system, these synapses were supplemented with AMPA-R. Whereas these studies provided exciting documentation of synapse formation and maturation during development, they did not demonstrate the recruitment of silent synapses during LTP in adult nervous systems. Subsequent studies using hippocampal slice preparations were able to show the conversion of silent, NMDA-R-only synapses to active, AMPA-R-containing synapses during LTP.<sup>(59)</sup>

Synapses in the CA1 region of the hippocampus could be silent because they possess AMPA-R that are inactive, or because they do not possess AMPA-R at all. Using immunolabeling techniques, several groups have shown the latter to be true: synaptic connections that have NMDA-R but lack AMPA-R are present both in cultured neurons and intact brain.<sup>(60–64)</sup> Indeed, AMPA-R can be directly observed in subsynaptic vesicular pools that cycle in and out of the synaptic membrane and that, upon LTP induction, fuse with overlying membranes to create active, AMPA-R-containing synapses.<sup>(65–72)</sup>

### CaMKII induces the delivery of AMPA receptors to synapses

If the conversion of silent synapses to active synapses in the hippocampus is an accurate model for LTP, then does CaMKII play a role in that conversion? CaMKII can phosphorylate the GluR1 subunit of AMPA-R on serine 831, and the induction of LTP results in the phosphorylation of this subunit in a CaMKII-dependent manner.<sup>(47–49)</sup> Such phosphorylation of AMPA-R results in an increase in their single channel conductance, which could contribute to the increase in synaptic efficacy seen in LTP.<sup>(73–75)</sup> However, these results do not explain the quantal nature of LTP, nor indicate a role for silent synapses or AMPA-R membrane cycling.

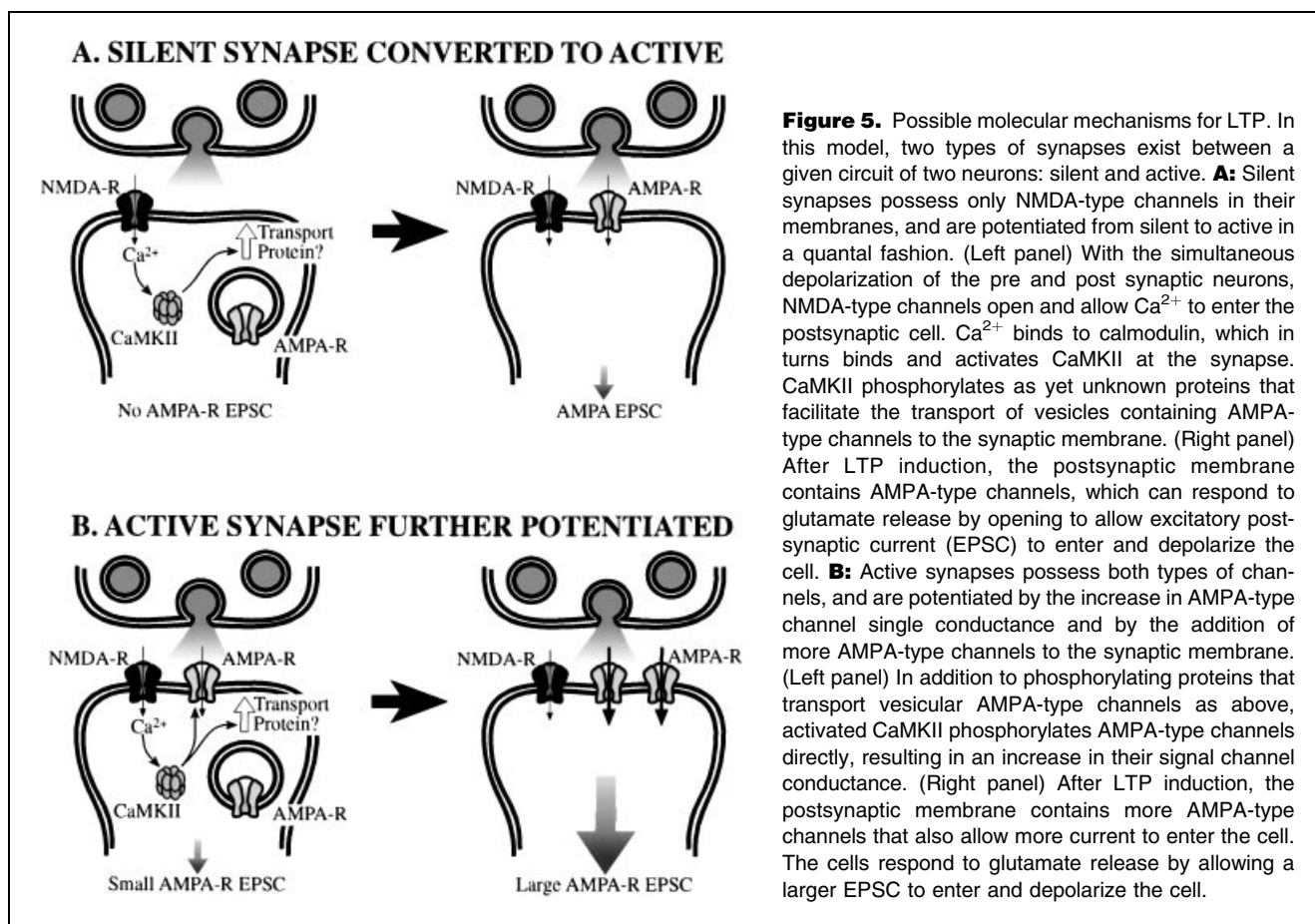
The first clues that CaMKII could regulate AMPA-R trafficking came from the glutamatergic synapses of the nematode *C. elegans*. An AMPA-type glutamate receptor subunit is encoded by the *glr-1* gene, and a GLR-1::GFP chimeric protein is localized to postsynaptic clusters along neurites of the nematode CNS.<sup>(76,77)</sup> *C. elegans* also has a single homologue of CaMKII, called UNC-43.<sup>(78)</sup> Nematodes that lack UNC-43/CaMKII have a decreased density of glutamatergic clusters and nascent GLR-1 accumulates in intracellular pools inside the neuron cell bodies, suggesting that UNC-43/CaMKII is needed to facilitate the transport of AMPA-R from the neuron cell body out to neurites for insertion at synaptic membranes.<sup>(79)</sup> Expression of a rat  $\alpha$ -CaMKII protein in the neurons of mutant nematodes that lack UNC-43

completely rescues the AMPA-R trafficking phenotype, suggesting that this function is conserved between the nematode and vertebrate proteins.

Malinow and colleagues have shown that CaMKII can drive AMPA-R into synapses in hippocampal neurons.<sup>(80)</sup> Using the Sindbis expression system, they expressed a GluR1::GFP chimeric protein, which they could distinguish electrophysiologically, in hippocampal slices along with a truncated, constitutively active CaMKII. Their results indicated that either LTP or increased CaMKII activity can induce the delivery of GluR1::GFP into synapses. Surprisingly, a serine-to-alanine mutation (S831A) that blocks phosphorylation of GluR1 by CaMKII has no effect on GluR1::GFP delivery, suggesting that phosphorylation of AMPA-R by CaMKII might only regulate the single channel conductance, and not AMPA-R trafficking. Instead, mutations in the GluR1 carboxy-terminus, which fits a predicted PDZ-domain binding site, blocked the AMPA-R trafficking effect of both increased CaMKII activity and LTP. These results suggest an elegant model whereby LTP activates CaMKII via  $\text{Ca}^{2+}$  influx from NMDA-R (Fig. 5A). CaMKII then phosphorylates target proteins that are involved in AMPA-R vesicular trafficking, including perhaps a PDZ-

domain protein. As a consequence, vesicles with AMPA-R fuse with overlying synaptic membranes at silent synapses, thereby supplementing these silent synapses with AMPA-R to convert them into active synapses. This would explain the increase in the number of AMPA-R quanta observed during LTP. At already active synapses, CaMKII could phosphorylate AMPA-R already present in the membrane, thereby increasing their single channel conductance (Fig. 5B). Moreover, active synapses could be supplemented with additional AMPA-R at the postsynaptic membrane. Both the increase in single channel conductance and the increased number of AMPA-R at the postsynaptic membrane could explain the overall increase in AMPA-R current amplitude that is sometimes observed during LTP.

AMPA-R form a tetrameric channel with more subunits than just GluR1 and, in the hippocampus, it is thought that most AMPA-R are composed either of GluR1–GluR2 heteromers or GluR2–GluR3 heteromers.<sup>(81)</sup> Surprisingly, the subunits do not behave the same way during AMPA-R trafficking. In the developing postnatal hippocampus, the predominantly expressed AMPA-R subunit is GluR4, which is delivered to synapses in an activity-dependent but CaMKII-independent



**Figure 5.** Possible molecular mechanisms for LTP. In this model, two types of synapses exist between a given circuit of two neurons: silent and active. **A:** Silent synapses possess only NMDA-type channels in their membranes, and are potentiated from silent to active in a quantal fashion. (Left panel) With the simultaneous depolarization of the pre and post synaptic neurons, NMDA-type channels open and allow  $\text{Ca}^{2+}$  to enter the postsynaptic cell.  $\text{Ca}^{2+}$  binds to calmodulin, which in turn binds and activates CaMKII at the synapse. CaMKII phosphorylates as yet unknown proteins that facilitate the transport of vesicles containing AMPA-type channels to the synaptic membrane. (Right panel) After LTP induction, the postsynaptic membrane contains AMPA-type channels, which can respond to glutamate release by opening to allow excitatory postsynaptic current (EPSC) to enter and depolarize the cell. **B:** Active synapses possess both types of channels, and are potentiated by the increase in AMPA-type channel single conductance and by the addition of more AMPA-type channels to the synaptic membrane. (Left panel) In addition to phosphorylating proteins that transport vesicular AMPA-type channels as above, activated CaMKII phosphorylates AMPA-type channels directly, resulting in an increase in their signal channel conductance. (Right panel) After LTP induction, the postsynaptic membrane contains more AMPA-type channels that also allow more current to enter the cell. The cells respond to glutamate release by allowing a larger EPSC to enter and depolarize the cell.

manner. Subsequently, GluR4 subunits are replaced with GluR2 subunits in an activity and CaMKII-independent manner.<sup>(82)</sup> Indeed, it appears that GluR2–GluR3 heteromers are continually being exchanged for other AMPA-Rs at synapses.<sup>(83)</sup> In contrast, GluR1–GluR2 heteromers are delivered to synapses in an activity-dependent manner that is subject to regulation by CaMKII and LTP. After GluR1–GluR2 heteromers are delivered to synapses in response to LTP, they eventually are replaced by GluR2–GluR3 heteromers, which are continually cycling and exchanging with the synaptic membrane. These two distinct, subunit-specific trafficking mechanisms appear to be regulated by different PDZ-domain proteins, which distinguish GluR1 and GluR2 subunits according to their carboxy-terminal sequences.<sup>(77,83)</sup> Such a mechanism could create an incredibly diverse repertoire of receptors at a given synapse, with each receptor being independently regulated by interactions with a specific PDZ-protein.

### Conclusions and future directions

Of course, a number of important questions remain unanswered concerning LTP, silent synapses, and CaMKII. Whether the predominant synaptic changes underlying LTP occur in the presynaptic cell, the postsynaptic cell, or both still remains as one of the largest question. For example, recent direct observation of presynaptic release using FM1-43 dyes suggests that such release in the CA3–CA1 pathway is increased in response to LTP.<sup>(58)</sup> Also controversial is whether the important contribution to potentiation during LTP by the postsynaptic cell is an increase in current amplitude from AMPA-R present at active synapses, the conversion of silent synapses to active synapses by the addition of AMPA-R to postsynaptic membranes, or some combination of these two mechanisms. If indeed synaptic efficacy is regulated by shuttling AMPA-R into and out of synaptic membranes, then how are the pools of “silenced” AMPA receptors organized? A number of steps in AMPA-R trafficking could be regulated, including subunit synthesis and assembly, ER to Golgi trafficking, neuron cell body to dendrite trafficking, mobilization of vesicular AMPA-R within dendritic spines, or some combination of all of these mechanisms. Although the proteins phosphorylated by CaMKII that regulate this process have not been identified, some excellent candidates are the PDZ-domain proteins. PDZ-domain proteins seem to play an important role both in the localization and membrane cycling of specific receptor subunit combinations, and appear to be important in the trafficking of GluR1–GluR2 heteromers in response to LTP and CaMKII activity. In *Drosophila*, CaMKII has been shown to control the localization of one of these PDZ-domain proteins, Discs large, by direct phosphorylation of Discs large protein.<sup>(84)</sup> Such a result raises the possibility that CaMKII regulates specific AMPA-R heteromers by directly phosphorylating the PDZ-domain proteins that bind to them.

The nature of the PDZ-proteins and the effect of phosphorylation on them will be interesting questions to be answered in the future.

Finally, I opened this review by describing the hippocampus not as a depository of memories but as a way-station through which memories are shuttled to the cortex, where they are consolidated into permanent memories. Whereas it is clear that CaMKII is needed in the hippocampus for long-term memory formation, how some of the suggested molecular models for CaMKII fit with models of the hippocampus as a memory way-station is not at all clear. One interesting observation that begins to address this issue is that mice that are heterozygous for a null mutation in CaMKII show normal learning and short-term memory, and normal hippocampal LTP; however, these mice show deficits in long-term memory and cortical LTP.<sup>(85)</sup> This would suggest that LTP and CaMKII are required for the consolidation of permanent memories in cortical networks, and that such memory consolidation in the cortex could share some of the molecular properties with memory formation in the hippocampus.

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