THE MOLECULAR BASIS OF CaMKII FUNCTION IN SYNAPTIC AND BEHAVIOURAL MEMORY

John Lisman, Howard Schulman‡ and Hollis Cline§*

Long-term potentiation (LTP) in the CA1 region of the hippocampus has been the primary model by which to study the cellular and molecular basis of memory. Calcium/calmodulin-dependent protein kinase II (CaMKII) is necessary for LTP induction, is persistently activated by stimuli that elicit LTP, and can, by itself, enhance the efficacy of synaptic transmission. The analysis of CaMKII autophosphorylation and dephosphorylation indicates that this kinase could serve as a molecular switch that is capable of long-term memory storage. Consistent with such a role, mutations that prevent persistent activation of CaMKII block LTP, experience-dependent plasticity and behavioural memory. These results make CaMKII a leading candidate in the search for the molecular basis of memory.

POSTSYNAPTIC DENSITY
An electron-dense thickening underneath the postsynaptic membrane at excitatory synapses that contains receptors, structural proteins linked to the actin cytoskeleton and signalling elements, such as kinases and phosphatases.

**Department of Biology, Brandeis University, Waltham, Massachusetts 02454, USA.*
‡Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305, USA, and SurroMed Inc., Mountain View, California 94043, USA.
§Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.
Correspondence to J.L.
e-mail: lisman@brandeis.edu

Calcium/calmodulin-dependent protein kinase II (CaMKII) is a Ca^{2+}-activated enzyme that is highly abundant in the brain, where it constitutes 1–2% of the total protein. The kinase is enriched at synapses and is the main protein of the POSTSYNAPTIC DENSITY (PSD) (FIG. 1). CaMKII is central to the regulation of glutamatergic synapses. This conclusion has emerged largely from the study of long-term potentiation (LTP), an activity-dependent strengthening of synapses that is thought to underlie some forms of learning and memory. At many excitatory synapses, LTP is triggered by Ca^{2+} entry into the postsynaptic cell. Several lines of evidence indicate that CaMKII detects this Ca^{2+} elevation and initiates the biochemical cascade that potentiates synaptic transmission.

But CaMKII might function as more than just a transducer during LTP induction; the enzyme might also be directly responsible for the persistence of LTP and therefore have a memory function. The strongest evidence for this idea comes from the fact that CaMKII remains activated for at least one hour after LTP induction — the longest period examined so far. Furthermore, autophosphorylation of threonine 286 is crucial for its persistent activation; a mutation that eliminates phosphorylation of this site blocks LTP. Although these results show the importance of persistent activity, it remains to be established for how long this activity is required. Persistence of limited duration might suffice if information were passed on to another, more persistent downstream process. However, computational studies show that the persistent activity of CaMKII could be very long-lived, indicating that it could serve as a molecular basis of long-term synaptic memory without any downstream process.

Progress in understanding the role of CaMKII has taken place at several levels. At the molecular level, there is now a better understanding of how autophosphorylation leads to persistent activity. Furthermore, recent studies show that CaMKII translocates to synapses, where it binds directly to the NMDA (*N*-methyl-D-aspartate) receptor. This translocation places the kinase in an ideal site to control synaptic strength; the molecular and structural processes by which this strengthening occurs are beginning to be unravelled. Progress has also been made in understanding how CaMKII contributes to brain function at the systems level. This is best exemplified by the observation that eliminating Thr286 phosphorylation not only blocks LTP, but also interferes

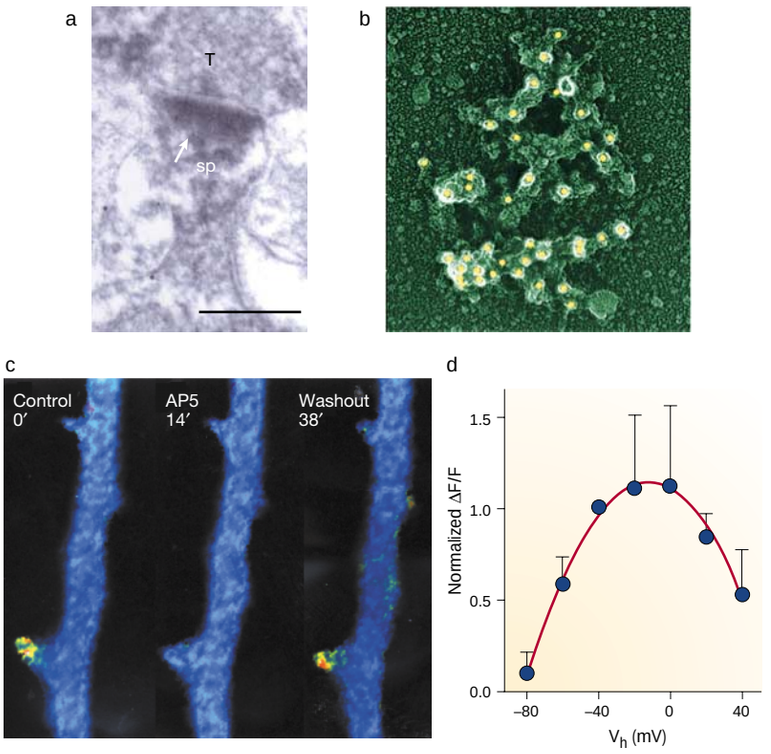

Figure 1 | **CaMKII in the postsynaptic density is ideally positioned to detect local Ca^{2+} entry through the NMDA receptor. a** | Immunohistochemical localization of calcium/calmodulin-dependent protein kinase II (CaMKII) shows labelling in a dendritic spine (sp), the site of glutamatergic synapses on CA1 pyramidal cells. Note the heavy labelling in the postsynaptic density (PSD; arrowhead). T, presynaptic terminal. Scale bar is 1 μm. **b** | Electron micrograph of negatively stained PSD isolated by subcellular fractionation. CaMKII is visualized by gold particles attached to a CaMKII antibody. **c** | Synaptic activation of NMDA (*N*-methyl-D-aspartate) receptors by a brief burst of synaptic activity causes local Ca^{2+} entry into a dendritic spine[126]. The fluorescence of calcium green, a Ca^{2+}-indicator dye, is rendered here in a pseudocolour, with red indicating the highest Ca^{2+} levels. NMDA antagonists (AP5 (D,L-2-amino-5-phosphonovaleric acid) in this case) block this entry, showing that the source is the NMDA receptor. **d** | Local Ca^{2+} entry measured with Ca^{2+}-sensitive dyes (quantified by the relative change in fluorescence) is highly enhanced by depolarization and could therefore account for the Hebbian character of long-term potentiation. V_h, holding potential. Part **a** courtesy of E. Jones; part **b** courtesy of T. Reese; part **d** adapted with permission from REF. 126 © 2000 Society for Neuroscience.

with experience-dependent plasticity *in vivo*. Indeed, behavioural tests show that memory is strongly impaired by this mutation. There is therefore little doubt that CaMKII is involved in the basic synaptic processes that store behaviourally relevant information.

We begin this review with a molecular description of CaMKII and its basic enzymatic function. We then discuss the evidence that CaMKII activation occurs during LTP and that this activation is necessary for LTP. We later examine the multiple mechanisms by which CaMKII enhances synaptic transmission, before turning to the question of how CaMKII acts as a molecular switch that is capable of storing long-term synaptic memory. In the last section, we consider the role of CaMKII in activity-dependent developmental processes, experience-dependent synaptic plasticity and memory. For a discussion of the basic properties of CaMKII and LTP, we refer the reader to previous reviews[1–5].

Structure and function of CaMKII

CaMKII comprises a family of 28 similar isoforms that are derived from four genes (α, β, γ and δ). The α- and β-subunits are the predominant isoforms in brain, where they form dodecameric holoenzymes that are composed of either one or both subunit types. Each isoform consists of a catalytic domain, an autoinhibitory domain, a variable segment and a self-association domain (FIG. 2a). The function of each of these regions is now well understood. The catalytic domain contains the ATP- and substrate-binding sites, as well as sites for interaction with anchoring proteins. This domain is inherently capable of catalysing the phosphotransferase reaction. Indeed, viruses encoding CaMKII that is truncated at the end of the catalytic domain have been used to express a monomeric fragment that is constitutively active. By contrast, the full-length form of the kinase has almost no catalytic activity under basal conditions because the autoinhibitory domain of each subunit inhibits its own catalytic domain (reviewed in REF. 2).

Elucidating the function of the autoinhibitory domain has provided detailed information on how the molecule can operate as a switch. There is a region within the autoinhibitory domain that resembles protein substrates (FIG. 2b). This pseudosubstrate region binds to the catalytic domain at the substrate-binding site (S site). The autoinhibitory domain can be thought of as a 'gate' that binds to the catalytic domain and inhibits enzyme activity (FIG. 2b). The gate is opened when Ca^{2+}/calmodulin binds to a region that overlaps with the pseudosubstrate region. Opening the gate activates the subunit, but also has a second important consequence: the exposure of Thr286 on the autoinhibitory domain (this numbering refers to the α-isoform and is Thr287 in other isoforms). When exposed, this site can be phosphorylated by a neighbouring subunit[6]. Importantly, once this site is phosphorylated, the gate cannot close, even after Ca^{2+} levels fall and Ca^{2+}/calmodulin dissociates from the enzyme[7–10]. The resulting persistent activity can be considered as a biochemical memory trace of the previous Ca^{2+} elevation. In this state, the enzyme is said to be autonomous and to show Ca^{2+}-independent activity.

The mechanism by which phosphorylation of Thr286 makes the enzyme autonomous has recently been determined. The gate binds to the catalytic domain at both the S site and a site known as T. Specifically, it is the region around Thr286 that binds to the T site, provided that Thr286 is not phosphorylated. T-site binding is required to position the pseudosubstrate sequence so that it inhibits the S site. After Thr286 becomes phosphorylated, binding to the T site cannot occur. As a result, the autoinhibitory domain cannot inhibit the S site and the kinase remains active[7] (FIG. 2b).

The other main parts of the kinase are the variable and the association regions. The association domain at the carboxy-terminal end of the kinase allows the assembly of a non-dissociable holoenzyme of 12 subunits. This region is linked to the catalytic and regulatory domains by a variable region that is responsible for most of the structural

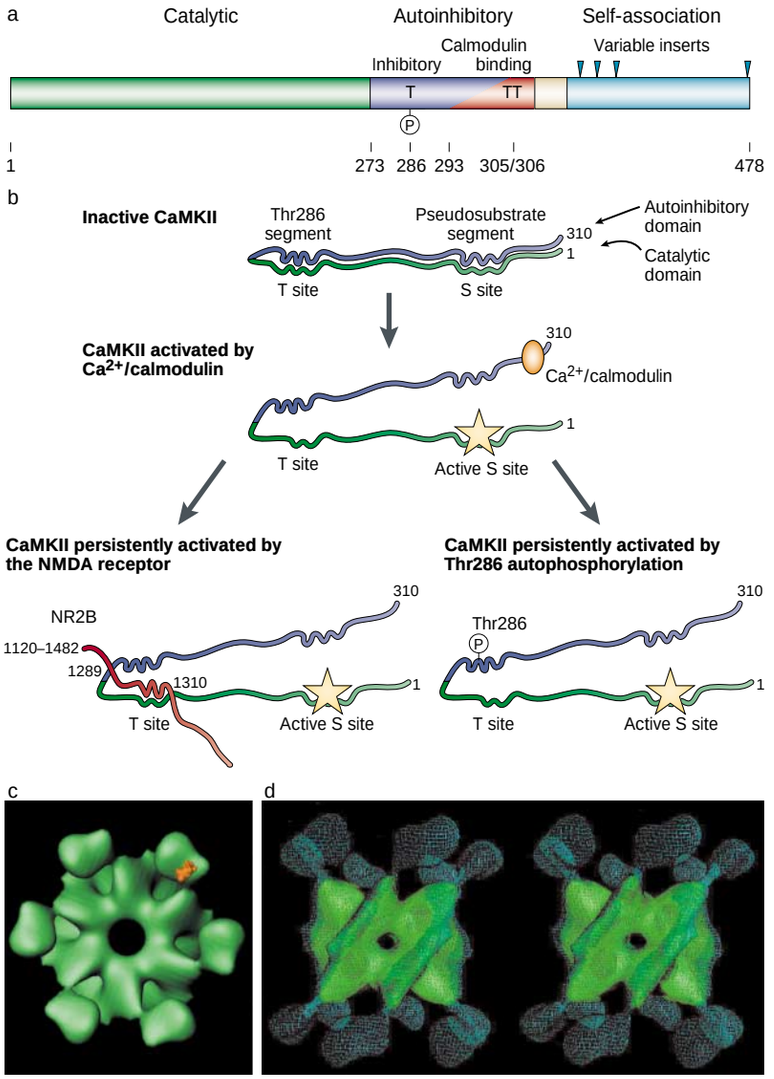

Figure 2 | **Structure and regulation of CaMKII. a** | The different functional domains in the primary structure of calcium/calmodulin-dependent protein kinase II (CaMKII). T represents threonine residues that are crucial phosphorylation sites. **b** | The autoinhibitory and catalytic domains form a gate that regulates activity. The enzyme is inhibited when the gate is closed because the autoinhibitory domain binds to the catalytic domain at the S and T sites (top). The binding of Ca^{2+}/calmodulin opens the gate and the enzyme becomes active (middle). A site on the NMDA (*N*-methyl-D-aspartate) receptor NR2B subunit can bind to the T side, keeping the gate open and the enzyme active even after the dissociation of calmodulin (bottom left). In the presence of Ca^{2+}/calmodulin, the Thr286 site can be phosphorylated by a neighbouring subunit. This is also sufficient to keep the gate open and the enzyme active even after dissociation of calmodulin (bottom right). **c** | Three-dimensional structure of CaMKII. This view shows only one of the hexameric rings formed by the catalytic regions of six subunits. **d** | Stereo view of CaMKII seen from a perspective perpendicular to that shown in **c**. The association domains of the 12 subunits form the gear-like structure. Part **d** reproduced with permission from REF. 13 © 2000 American Society for Biochemistry and Molecular Biology.

ALTERNATIVE SPLICING
During splicing, introns are excised from RNA after transcription and the cut ends are rejoined to form a continuous message. Alternative splicing gives rise to different messages from the same DNA molecule.

differences between isoforms. In this region, sequences of 9–127 amino acids are inserted by ALTERNATIVE SPLICING. These inserts can direct the targeting of the kinase to specific intracellular sites and modify the sensitivity to Ca^{2+}/calmodulin.

CaMKII is large enough to be visualized by electron microscopy[11,12]. Three-dimensional reconstructions of the kinase reveal considerable detail about the arrangement of subunits[13]. As shown in FIG. 2c,d, the catalytic/regulatory domains of the 12 subunits form two hexameric rings. Each of these domains is linked through a narrow stalk to the central mass of the molecule. This mass is composed of the 12 association domains, which are assembled into a gear-shaped structure that is formed by six slanted flanges. Looking down or from the sides, there are holes in the gear structure. The meaning of most aspects of this structural design remains unclear. The ring structure, however, seems directly related to the memory function of the kinase, as we will discuss later.

Different activation states of CaMKII. FIGURE 3 shows that CaMKII can be activated to different degrees, with decay times that depend on the magnitude of the Ca^{2+} signal and the properties of the phosphatases that dephosphorylate the kinase. Weak signals can activate the kinase without causing autophosphorylation or persistent activation. In this case, the subunits that bind Ca^{2+}/calmodulin will become inactive within 0.1–0.2 s after the Ca^{2+} level falls, the time constant for calmodulin dissociation from the kinase (FIG. 3a).

If the duration or magnitude of the Ca^{2+} elevation is greater, autophosphorylation will occur. The requirement for initiating this reaction is that two molecules of Ca^{2+}/calmodulin bind to two subunits on the same holoenzyme. Two molecules of Ca^{2+}/calmodulin are required because one binds to a given subunit and activates it, while the second one binds to a neighbouring subunit, causing a conformational change that 'presents' Thr286 to its neighbour for phosphorylation. Once one site on a ring is phosphorylated, the propagation of phosphorylation around the ring can proceed more easily, probably in a directional process. Propagation can occur in response to lower levels of Ca^{2+} than initiation, because propagation requires binding of a single Ca^{2+}/calmodulin to a subunit adjacent to the one that is already phosphorylated[6]. Once phosphorylated, CaMKII will remain active when Ca^{2+} returns to basal levels until the kinase is dephosphorylated (FIG. 3b). There seem to be pools of CaMKII that become dephosphorylated on a timescale of several minutes[14]. This form of short-term persistent activation might have an important functional role by extending kinase activity significantly beyond the period of Ca^{2+} elevation. Short-term persistence depends in a graded way on the number of phosphorylated subunits, and can therefore integrate multiple Ca^{2+} pulses over time, provided that they fall within the integration window that is determined by the time constant of dephosphorylation. These properties make the kinase able to function as a frequency detector[15].

Long-term persistent activation (FIG. 3c) occurs after the induction of LTP (see below). Theoretical analysis indicates that this form of activation occurs because the group of CaMKII molecules in the special chemical environment of the PSD acts as a bistable switch. A switch of this kind turns on when a threshold number of kinase sites are phosphorylated. The 'on' state of the

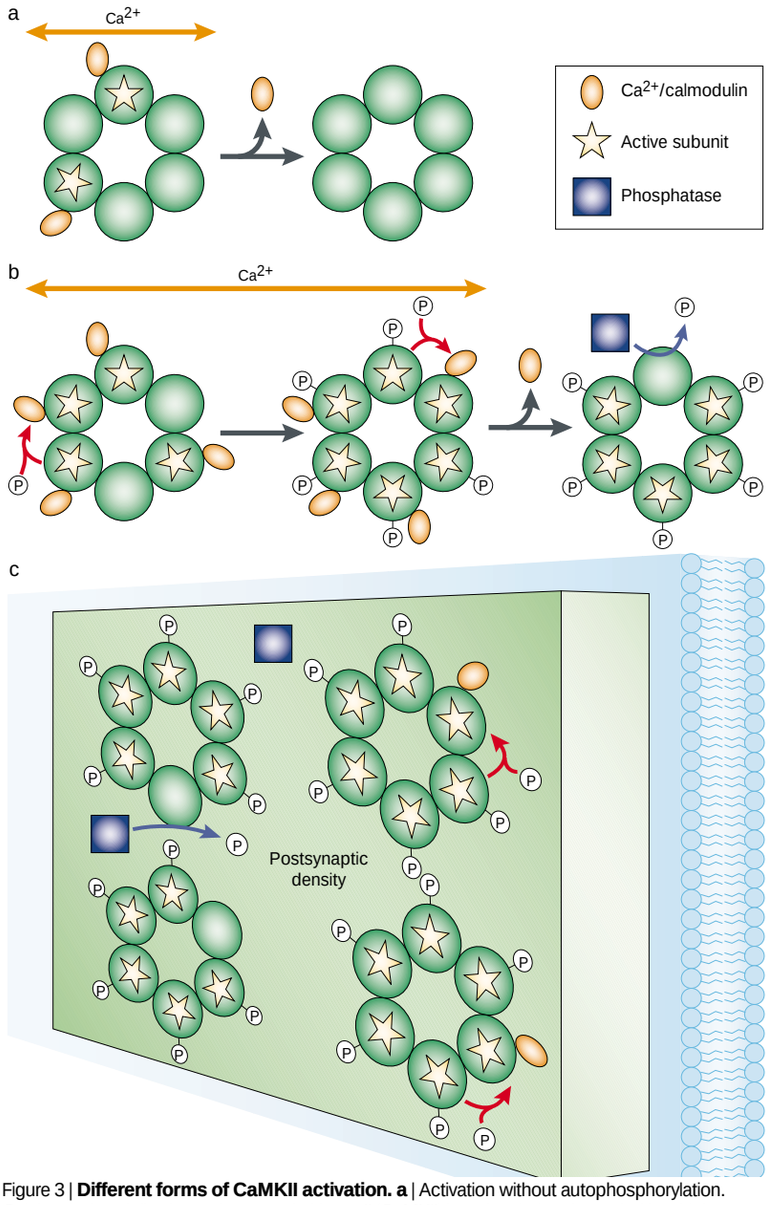

Figure 3 | **Different forms of CaMKII activation. a** | Activation without autophosphorylation. Calcium/calmodulin-dependent protein kinase II (CaMKII) is active during the period in which Ca^{2+}/calmodulin is bound, but little or no autophosphorylation occurs with brief or weak stimuli because the occurrence of neighbouring subunits with bound Ca^{2+}/calmodulin is low. Calmodulin dissociates within less than 1 s after Ca^{2+} levels fall. **b** | Short-term persistent activation by autophosphorylation of threonine 286. Activity persists after Ca^{2+} falls, but declines if Thr286 becomes dephosphorylated. Pools of CaMKII exist in which this occurs in minutes. **c** | Long-term persistent activation when the rate of autophosphorylation exceeds the rate of dephosphorylation. This form of activation could occur in the special environment of the postsynaptic density (PSD) through the interaction of protein phosphatase 1 (PP1) with multiple phosphorylated holoenzymes. The blue square represents PP1, which is bound to the PSD and can dephosphorylate CaMKII.

HEBB'S RULE
"When the axon of cell A excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased."

switch can last for very long periods, because the kinase acts faster than the PSD phosphatase on Thr286 sites. This binary transition might account for the all-or-nothing nature of LTP induction, which becomes evident when single synapses are examined[16]. The mechanism and role of long-term persistent activation will be discussed extensively in subsequent sections of this review.

CaMKII activation and LTP induction

CA1 hippocampal synapses have served as a model system by which to understand synaptic plasticity. Induction of LTP at these synapses occurs according to HEBB'S RULE: a synapse is strengthened if there is repeated coincident activity in both the presynaptic and postsynaptic cells. According to neural network theory, this property makes it possible for networks to store associative memories. One of the main advances in the study of LTP has been the demonstration that the NMDA receptor can act as a detector of coincident activity in the pre- and postsynaptic cells. The channels formed by these receptors open efficiently only when glutamate is released from the presynaptic terminal and the postsynaptic cell is strongly depolarized (FIG. 1d). Channel opening produces a rise in Ca^{2+} that is largely restricted to the dendritic spine onto which the active synapse terminates (FIG. 1c). This Ca^{2+} elevation is both necessary and sufficient for LTP induction (see REFS 4,5 for reviews).

Many studies have shown that CaMKII is activated by the NMDA-receptor-mediated Ca^{2+} elevation that occurs during LTP induction. It was initially found that Ca^{2+}-independent CaMKII activity is persistently elevated for at least one hour after LTP induction[17] (FIG. 4a). Subsequent work showed that this activation was accompanied by phosphorylation of both the α- and β-subunits[18], and that phosphorylation occurred on Thr286/287 (REF. 19; FIG. 4b). The increase in activity is small (~15%), but this is not surprising given the small fraction of synapses that is stimulated during LTP induction. Although there was initial concern that the increase might be related to the synthesis of further CaMKII after LTP induction[20], it was later shown that the enhancement also took place if protein synthesis was blocked[21].

Is the increase in kinase activity restricted to stimulated synapses? This would be required to account for the fact that LTP occurs preferentially at stimulated synapses, a property known as synapse specificity. As action potentials are generated during LTP induction and can open voltage-dependent Ca^{2+} channels throughout the cell[22], it is not surprising that a fraction of activated CaMKII is spatially diffuse[20]. However, analysis at higher spatial resolution indicates that highly localized CaMKII activation can also occur[23], as expected from the existence of Ca^{2+} hotspots (FIG. 1c). Recent work shows that there is an increase in CaMKII content and CaMKII activity after LTP induction within the PSD itself[24,25]. Together, these results indicate that LTP is accompanied by the persistent activation of CaMKII, some of which occurs specifically at synapses.

CaMKII is necessary for LTP induction. Testing whether CaMKII is required for LTP induction was made possible by the development of specific inhibitors of this kinase[26]. Peptides modelled after the autoinhibitory region (for example, autocamtide-2 or a peptide comprising residues 273–302 of CaMKII) block both Ca^{2+}-dependent and Ca^{2+}-independent activity of the enzyme without interfering with other calmodulin-dependent processes. Membrane-permeant inhibitors of CaMKII,

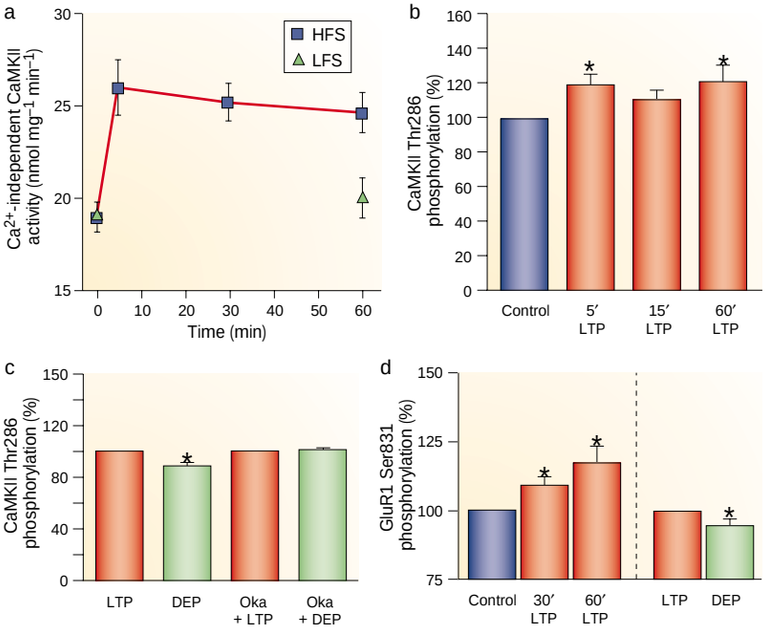

Figure 4 | **CaMKII and its substrate GluR1 are phosphorylated after hippocampal LTP and dephosphorylated after depotentiation. a** | The Ca^{2+}-independent, persistent enzymatic activity of calcium/calmodulin-dependent protein kinase II (CaMKII) is enhanced after high-frequency stimulation (HFS) that induces long-term potentiation (LTP). The same number of stimuli given at low frequency (LFS) does not induce LTP and has no effect on kinase activity. **b** | LTP enhances the phosphorylation of threonine 286, measured using an antibody that is specific for this phosphorylated site at various times after LTP induction. **c** | Depotentiation (DEP) produces a decrease in CaMKII phosphorylation at Thr286 (left) that is blocked by the phosphatase inhibitor okadaic acid (Oka). **d** | Persistent enhancement of the CaMKII-dependent phosphorylation of serine 831 on ionotropic glutamate receptor subunit GluR1 at various times after LTP induction (left). This is reversed by depotentiation (right). Part **a** adapted with permission from REF. 17 © 1993 American Society for Biochemistry and Molecular Biology; part **b** adapted with permission from REF. 19 © 1997 American Association for the Advancement of Science; part **c** adapted with permission from REF. 84 © 2001 American Society for Biochemistry and Molecular Biology; part **d** adapted with permission from *Nature* (REF. 57) © 2000 Macmillan Magazines Ltd.

such as KN62 and KN93, block the Ca^{2+}-dependent activity of the enzyme by interfering with calmodulin binding[27], and prevent LTP induction by brief TETANIC STIMULATION — a standard LTP-inducing protocol.

The depolarization level that is needed to activate NMDA receptors requires the summation of multiple synaptic inputs and the generation of dendritic spikes. It is therefore possible that CaMKII antagonists block LTP because they interfere with postsynaptic depolarization rather than with events downstream of the NMDA receptor. To determine whether these 'core' downstream processes are indeed affected by CaMKII inhibition, a different LTP-inducing method has been used: a PAIRING PROTOCOL in which low-frequency stimulation is paired with artificially imposed membrane depolarization. LTP produced by this protocol is also completely blocked by CaMKII inhibitors[28] (FIG. 5a), indicating that the kinase is indeed involved in a core process.

As with any pharmacological approach, the specificity of inhibitors cannot be assured (CaMKI and CaMKIV are probably also blocked by CaMKII inhibitors), and a complementary genetic approach is therefore needed. Consistent with the pharmacological results, knocking out α-CaMKII in mice reduced the magnitude of LTP. The residual LTP found in these animals (~50%) might reflect the fact that the total CaMKII activity was reduced by only 45%; the remaining activity presumably corresponded to β-CaMKII[29,30].

A much more complete abolition of LTP was subsequently achieved by an α-CaMKII mutation that involved a single amino-acid modification[31] — the replacement of Thr286 by alanine. This form of the kinase can undergo Ca^{2+}-dependent activation, but it cannot become persistently active (FIG. 3). LTP produced by a pairing protocol in these animals was almost completely blocked (FIG. 5b). As this single-site mutation was more powerful in blocking LTP than the knockout, this effect could be explained by a DOMINANT-NEGATIVE action in which mutant subunits disrupt the propagation of phosphorylation around the hexameric ring (FIG. 3c). The small residual component of LTP could in this case be related to the activity of protein kinase C (PKC)[32–34]. Together, the pharmacological and genetic evidence strongly argues that a large fraction of LTP is dependent on CaMKII and that this enzyme is involved in a core process of LTP.

CaMKII is sufficient to induce LTP. There is strong evidence that LTP involves a postsynaptic process, which selectively enhances AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-receptor-mediated transmission[5]. One line of evidence that supports this conclusion is that LTP enhances the response to applied glutamate[35,36]. This enhancement can be mimicked by the postsynaptic application of CaMKII, which produces a two- to threefold increase in the response to glutamate analogues[37,38]. This effect also occurs in hippocampal slices when CaMKII is introduced by either intracellular perfusion or viral expression[38,39]. The response to synaptically released glutamate is also increased two- to threefold by the kinase.

Does CaMKII affect synaptic transmission in the same way as LTP? In other words, does CaMKII activity mimic LTP by using an independent mechanism or does it affect the same processes as LTP? Several methods have been used to address this question. For example, QUANTAL ANALYSIS has shown that LTP induction produces an increase in QUANTAL SIZE and a decrease in FAILURE RATE[40,41]. This decrease in failures probably reflects the fact that, before LTP, some vesicles were released at 'silent' synaptic contacts. After LTP, these SILENT SYNAPSES are made functional by the addition of AMPA receptors[42]. Perfusion of activated CaMKII into CA1 cells mimics the effects of LTP on quantal parameters: it increases quantal size, as measured by the amplitude of spontaneous excitatory postsynaptic currents (EPSCs)[38], and decreases the probability of failures (FIG. 6a,b).

Another strategy to test whether CaMKII and LTP affect synaptic efficacy by the same mechanisms has been to explore whether the two stimuli occlude each other[38,39]. Synapses that are potentiated by CaMKII cannot undergo LTP. Conversely, synapses that have undergone LTP are virtually insensitive to CaMKII (FIG. 6c).

TETANIC STIMULATION
A train of stimuli in which afferent axons are briefly activated at high frequency. In LTP experiments, a 1-s train of pulses delivered at a frequency of 100 Hz is commonly used to potentiate transmission.

PAIRING PROTOCOL
If a cell is artificially depolarized while low-frequency stimulation is delivered, synaptic transmission will be potentiated because the depolarization relieves the Mg^{2+}-dependent block of NMDA receptors.

DOMINANT NEGATIVE
A mutant molecule that can form a heteromeric complex with the normal molecule, knocking out the activity of the entire complex.

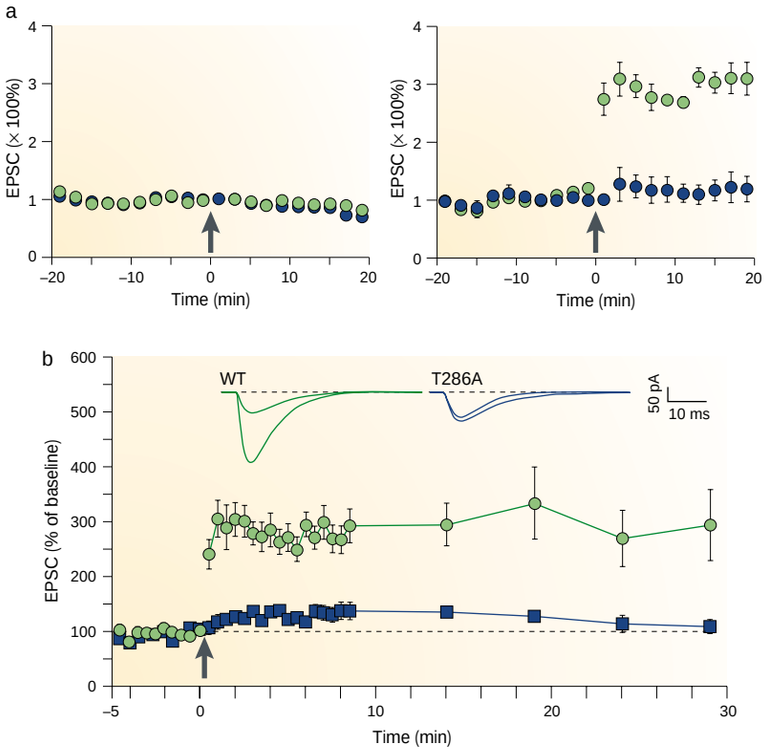

Figure 5 | **LTP induced by a pairing protocol requires CaMKII activity. a** | Long-term potentiation (LTP) is blocked by intracellular perfusion of a calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor peptide (left; arrow), but not by a control peptide (right). Results from the experimental pathway are shown in green; those from a control pathway that was not stimulated during the induction protocol are shown in blue. **b** | LTP is strongly reduced in an animal that expresses a form of CaMKII in which threonine 286 is replaced by alanine (blue). Insets show excitatory postsynaptic currents (EPSCs) before and after LTP induction. WT, wild type. Part **a** adapted with permission from REF. 28 © 1997 Society for Neuroscience; part **b** adapted with permission from REF. 31 © 1998 American Association for the Advancement of Science.

These results make a strong case for the proposal that CaMKII can potentiate transmission and that the mechanisms involved are the same as those recruited during LTP induction.

Mechanisms of CaMKII-mediated potentiation

Rapid progress has been made in understanding the multiple mechanisms by which AMPA-receptor-mediated transmission is strengthened during LTP and the specific role of CaMKII in these mechanisms. One of them is a classic modulatory reaction in which CaMKII phosphorylates AMPA receptors that are already localized at synapses, enhancing their conductance. However, recent work indicates the existence of other mechanisms that involve changes in the composition of the synapse. In particular, both CaMKII and AMPA receptors are added to the synapse during LTP. Understanding this structural assembly will require the elucidation of processes that bring these molecules to the synapse and hold them there.

Translocation to PSD and NMDA receptor binding. The use of CaMKII labelled with the green fluorescent protein (GFP) has made it possible to visualize CaMKII in living cells and to observe its movement. Under resting conditions, F-actin binds to the β-subunit of CaMKII and holds it away from synapses. Ca^{2+} elevation causes the kinase to dissociate from actin in an autophosphorylation-independent manner[43], allowing it to diffuse to the synapse. Indeed, electron microscopic studies show that raising Ca^{2+} causes a build-up of CaMKII in the PSD[44].

Recent work indicates that a binding partner for CaMKII is the NMDA receptor within the PSD. Activation, either alone[45] or when followed by autophosphorylation[45–48], increases the association of α-CaMKII and β-CaMKII with the cytoplasmic carboxy-terminal domain of the NMDA receptor NR2B subunit. The kinase also associates with the NR1 subunit, an association that is enhanced by autophosphorylation[48]. So, as autophosphorylation occurs, the kinase binds to multiple sites on the NMDA receptor and gradually becomes more strongly bound.

Surprisingly, binding of CaMKII to the NMDA receptor regulates kinase activity[45]. After a subunit binds to NR2B, it remains active even after the dissociation of Ca^{2+}/calmodulin (FIG. 2b). This form of activation does not require autophosphorylation. Instead, it arises because the sequence on NR2B to which the kinase anchors has a striking homology to the autoinhibitory domain that binds to the T site (FIG. 2b). NR2B can also bind to the T site, acting as a 'wedge' in the autoinhibitory gate, keeping it open even after dissociation of Ca^{2+}/calmodulin. This active state is transient, lasting from seconds to minutes, but is likely to have important consequences. First, unlike autonomous activity generated by autophosphorylation, this activation cannot be reversed by phosphatase activity. Second, binding of an unphosphorylated subunit to the NMDA receptor produces 'trapping': a greatly enhanced affinity of calmodulin for the kinase. Trapping could keep the kinase at the synapse because it prevents a secondary autophosphorylation of the calmodulin-binding domain (Thr305) that might otherwise speed dissociation of the kinase from synaptic sites[49]. Third, activation of the subunit bound to the NMDA receptor would facilitate further autophosphorylation around the ring, because binding of just one Ca^{2+}/calmodulin to a neighbouring subunit would be sufficient to produce autophosphorylation[45]. In this sense, binding of the NMDA receptor to CaMKII can be considered as a catalyst that promotes further autophosphorylation, which, in turn, strengthens kinase binding to the NMDA receptor.

Although there has been rapid progress in our understanding of the binding of CaMKII to the PSD, it is also clear that much remains to be clarified. First, CaMKII binds to at least two other PSD proteins[47,50] — densin-180 and α-actinin 4. Autophosphorylation of CaMKII does not seem to be as important for these interactions as for binding to the NMDA receptor. Second, *in vitro* studies show that, depending on the conditions, phosphatase activity may[51] or may not[25] promote dissociation of the CaMKII from the PSD, illustrating the complexity of their interaction. CaMKII association with the PSD is reversible under some conditions[44,49], but translocation was stimulated in these experiments by elevating Ca^{2+}

QUANTAL ANALYSIS
This type of analysis was developed to account for the properties of transmitter release at the neuromuscular junction. It aims to describe release as a function of three basic parameters: the number of release sites (n), the probability of release at each site (p), and the postsynaptic response elicited by a single transmitter vesicle (q). The amplitude of a synaptic event can be described by the product npq. Although quantal analysis provides a valid account of release at the neuromuscular junction, some of its underlying assumptions might not be valid at central synapses.

QUANTAL SIZE
The synaptic response elicited by a single vesicle of transmitter as determined by postsynaptic factors such as the number and affinity of receptors.

FAILURE RATE
The probability that a presynaptic action potential will fail to produce a postsynaptic response.

SILENT SYNAPSE
A synapse that contains NMDA receptors but no AMPA receptors and is therefore functionally silent during low-frequency, basal synaptic transmission.

under conditions that did not evoke LTP. An unanswered question is whether translocation is persistent under conditions in which LTP is induced. Understanding translocation could help to explain why PSD thickness and CaMKII content gradually increase during development[52] — processes that can be blocked by NMDA antagonists[53]. Despite the remaining uncertainties, the emerging picture is clear: autophosphorylation of CaMKII leads to tight binding of the kinase to the NMDA receptor, placing it in a strategic position to control synaptic function. The enzymatic and structural processes by which CaMKII can strengthen synaptic transmission are discussed next.

Phosphorylation of GluR1. The AMPA receptor subunit GluR1 contains a single phosphorylation site — serine 831 — that, when phosphorylated by CaMKII, enhances channel function[54,55]. Studies on homomeric GluR1 receptors[56] show that the unphosphorylated channel has conductance states of 9, 14, 20 and 28 pS. When active CaMKII is expressed with GluR1, the same conductances are observed, but the probability of transitions to the high-conductance states is raised, increasing the effective conductance by about 50%. Replacing Ser831 with aspartate, the negative charge of which mimics the presence of a phosphate group, can reproduce this effect.

Biochemical studies have shown that receptor phosphorylation occurs during LTP, as its induction increases ^{32}P incorporation into the GluR1 subunit[19]. Phosphorylation was blocked by NMDA antagonists and by the CaMKII antagonist KN62. The effect of KN62 is important, because it indicates that phosphorylation is not due to PKC, an enzyme that can phosphorylate Ser831 *in vitro*. With the development of specific antibodies against GluR1 phosphorylated at Ser831, it became possible to show that LTP increased receptor phosphorylation 30 minutes after LTP induction, with a further increase by one hour[57] (FIG. 4d).

The phosphorylation of Ser831 after LTP induction would be expected to increase the AMPA channel conductance, and this has been directly observed[58]. In some cells, the magnitude of this increase is sufficient to account for LTP. But in others, LTP occurs without a change in conductance, indicating that there must be other mechanisms of potentiation besides phosphorylation. This idea is supported by the fact that other LTP-induction methods (such as pairing protocols) produce an increase in transmission that is much larger than could be accounted for by the increase in conductance. The existence of other potentiation mechanisms has been confirmed by recent work[59] showing that CaMKII can potentiate transmission after the elimination of Ser831.

Addition of AMPA receptors to synapses. The addition of AMPA receptors to silent synapses seems to be an important component of LTP. Immunolabelling experiments directly show the existence of synapses that lack AMPA receptors[60]. It has also been possible to observe that silent synapses become functional after LTP induction[43], and to show that new receptors are added to synaptic contacts[59]. So, there is little doubt

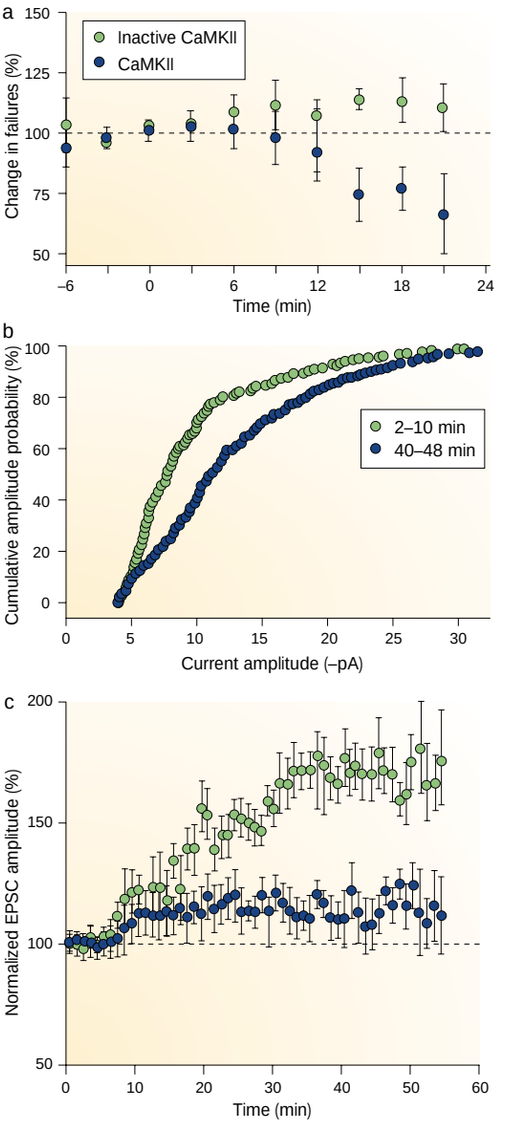

Figure 6 | **Effects of perfusion of active CaMKII on CA1 pyramidal cells. a** | Active calcium/calmodulin-dependent protein kinase II (CaMKII) reduces the number of postsynaptic failures. Minimal stimulation was used to activate only a few presynaptic axons. **b** | Active CaMKII increases the amplitude of spontaneous synaptic potentials after the enzyme has time to diffuse from the cell body to the dendrites. Plots are cumulative histograms. **c** | If long-term potentiation (LTP) is induced in one pathway (blue), but not in another (green), subsequent perfusion of CaMKII enhances transmission much more strongly in the pathway that had not undergone LTP. This shows occlusion between CaMKII-induced potentiation and LTP induction. Adapted with permission from REF. 38 © 1995 National Academy of Sciences, USA.

that the decrease in failures after LTP induction is due, at least in part, to the addition of AMPA receptors to silent synapses. As discussed above, CaMKII application also reduces the probability of synaptic failures[38] (FIG. 6a), indicating that CaMKII might convert silent synapses into functional contacts. This conclusion is

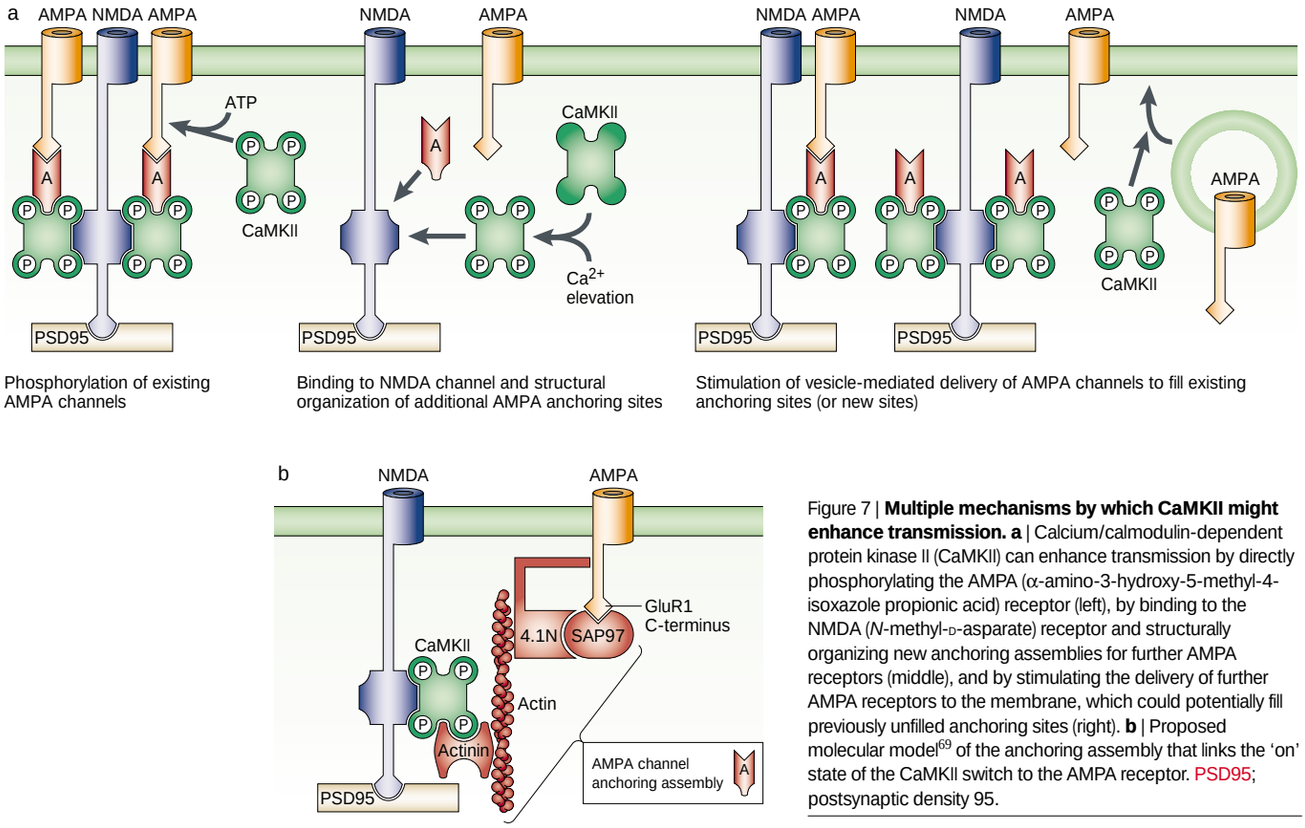

Figure 7 | **Multiple mechanisms by which CaMKII might enhance transmission. a** | Calcium/calmodulin-dependent protein kinase II (CaMKII) can enhance transmission by directly phosphorylating the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (left), by binding to the NMDA (*N*-methyl-D-asparate) receptor and structurally organizing new anchoring assemblies for further AMPA receptors (middle), and by stimulating the delivery of further AMPA receptors to the membrane, which could potentially fill previously unfilled anchoring sites (right). **b** | Proposed molecular model[69] of the anchoring assembly that links the 'on' state of the CaMKII switch to the AMPA receptor. PSD95; postsynaptic density 95.

supported by experiments in which a constitutively active form of CaMKII was overexpressed in the tectal cells of living frogs using a viral vector. Whereas ~50% of synapses in immature regions of the tectum are normally silent, this figure was reduced to ~10% by CaMKII (see below)[61].

The mechanisms that control the number of AMPA receptors at synapses are the subject of intense investigation. Regulation seems to occur at two levels: the trafficking process that modulates the insertion and removal of receptors from the plasma membrane, and the anchoring processes that hold receptors at the synapse. Strong evidence for a distinction between trafficking and anchoring is provided by two recent experiments. First, transfection of cultured cells with a form of the protein STARGAZIN that is defective in PDZ interactions produces a marked reduction in synaptic glutamate receptors without affecting extrasynaptic receptors[62]. Second, receptors that contain GluR1 are inserted extrasynaptically and then slowly captured at synaptic sites[63]. In the next sections, we consider separately the mechanisms by which CaMKII triggers AMPA receptor insertion into the plasma membrane and their capture at synaptic sites.

AMPA receptor trafficking. Under resting conditions, vesicles that harbour AMPA receptors are contained in the dendrite and somehow excluded from spines. After NMDA receptor activation, AMPA receptors can move into spines[64] and seem to enter to the plasma membrane by exocytosis[65], a process that depends on the identity of the subunit involved[66]. A direct assay for GluR1 delivery by exocytosis is still lacking. So, our best view of this process comes from the examination of how activity stimulates general exocytosis in dendrites using FM1-43, a lipophilic dye that is taken up by synaptic vesicles during endocytosis[67]. Exocytosis was blocked by CaMKII inhibitors and could be stimulated by constitutively active CaMKII in the absence of Ca^{2+}. The molecular mechanisms by which CaMKII stimulates exocytosis — and, in particular, the exocytosis of AMPA-receptor-containing vesicles — remain to be determined.

AMPA receptor anchoring. CA1 synapses differ markedly in their size and in the number and density of AMPA receptors that they possess[60]. As we have seen, CaMKII can translocate to the PSD and bind to NMDA receptors. In a recent study, both of these changes were observed simultaneously after the stimulation of NMDA receptors[68]. It has been proposed that these phenomena are directly linked because the stable binding of autophosphorylated CaMKII to the NMDA receptor organizes a structural process that leads to the incorporation of AMPA-receptor-binding proteins into the PSD, and to the subsequent anchoring of additional AMPA receptors[69] (FIG. 7a).

A structural mechanism by which CaMKII could organize an AMPA-receptor-anchoring assembly[69] is shown in FIG. 7b. This model is based on recently discovered binding interactions within the PSD. Actin

STARGAZIN
A molecule that is involved in the delivery of AMPA-type glutamate receptors to the cell surface. Mice with mutations in this protein — the so-called stargazer mice — arose spontaneously and were detected by their distinctive head-tossing motion and unsteady gait.

filaments are assumed to provide a link between a CaMKII/actinin complex and the complex formed by protein 4.1 and SAP97, two GluR1-binding proteins. F-actin can link these two complexes because it binds to both protein 4.1 and actinin. Consistent with this view, actin is important in localizing AMPA receptors at synapses; depolymerization of actin leads to a rapid reduction in AMPA-receptor-mediated transmission, to loss of synaptic AMPA receptors, and to a reduction in LTP. These phenomena occur without any gross change in spine morphology or any substantial alteration in NMDA-receptor-mediated synaptic transmission[70,71]. All of the proteins that feature in the model exist in the PSD, and their binding interactions have been shown directly (see REF. 72 for review).

The model addresses fundamental issues of synaptic design. First, the structural role assigned to CaMKII would explain why its concentration in the PSD is so high. Investigators have long pondered why this should be so if its function is solely enzymatic. Second, a fundamental requirement for synapses involved in memory storage is that synaptic strength be bidirectionally modifiable. Because binding of CaMKII to the NMDA receptor is enhanced by CaMKII phosphorylation, the structurally mediated strengthening could be reversed by dephosphorylating CaMKII through the phosphatase-dependent processes (FIG. 4c,d) that are involved in DEPOTENTIATION[73]. Last, the model posits a clear separation of the storage processes that underlie synaptic memory from dynamic processes that control the number of AMPA receptors at the synapse. Storage is mediated by the phosphorylation state of the CaMKII switch, but does not depend on receptor trafficking. The fraction of AMPA-anchoring sites that are filled can be continuously regulated by trafficking processes[74]. This separation has the advantage of making stored information insensitive to the vicissitudes of receptor trafficking.

The multiple mechanisms by which CaMKII can affect AMPA-receptor-mediated transmission are summarized in FIG. 7a. Strengthening might involve phosphorylation of existing receptors, insertion of further AMPA receptors into existing, but unfilled anchoring sites, or creation and filling of new anchoring sites. The redundancy of these mechanisms could confer robustness to LTP expression. So, if trafficking were affected, LTP expression could still occur by the direct phosphorylation of existing receptors. Similarly, regulation of the number of anchoring sites could lead to LTP even if AMPA-receptor-phosphorylation were disabled. The redundancy of mechanisms would predict that a mutation that interferes with just one of these processes would have less effect on LTP than mutations that interfere with CaMKII. An interesting possibility is that different pools of CaMKII are coupled to different expression mechanisms[75].

DEPOTENTIATION
A reversal of LTP by low-frequency synaptic stimulation. Depotentiation shares some characteristics with long-term depression; both are induced by low-frequency stimulation, and both require NMDA receptor and protein phosphatase activity. However, it is unclear whether they represent the same phenomenon or are fundamentally different.

CaMKII as a molecular switch

Neurons have thousands of synapses, each of which is thought to be independently modifiable by LTP. It is precisely this specificity that makes it possible for neurons to store large amounts of information, making synaptic modification an attractive mechanism for memory storage. Given that each synapse is independently modifiable, it is unlikely that information storage could rely on transcriptional signals in the nucleus. This idea prompted interest in the question of whether protein interactions could subserve long-term information storage through local processes that are confined to each synapse[76,77]. One solution to this problem is the idea of a kinase switch[77], the function of which was first formulated in general terms. It was suggested that a stable 'on' state could occur if stimulus-induced phosphorylation of the kinase made it active, even after the stimulus was removed. In this 'on' state, the kinase could phosphorylate itself, thereby producing an autocatalytic reaction. In addition, it was proposed that the phosphatase that dephosphorylates the kinase becomes saturated when the kinase is highly phosphorylated, allowing the kinase to rephosphorylate sites faster than the phosphatase can dephosphorylate them. Furthermore, as the function of this bistable switch is dependent on the pool of interacting kinase and phosphatase molecules, the informational state of the switch could potentially be stable despite the turnover of individual molecules.

So, it is possible, in principle, to store long-term information locally at synapses using a kinase switch. After it was discovered that CaMKII has a stable 'on' state and can phosphorylate itself, specific switch models were formulated using the specific properties of CaMKII[78]. As we discuss next, these models have undergone important revisions as more has been learned about the kinase and phosphatase reactions[69,79].

Recent findings indicate that the dephosphorylation of CaMKII in the PSD is very different from that in the cytoplasm. Purified CaMKII can be dephosphorylated by either protein phosphatase 1 (PP1) or PP2A. In the cytoplasm, CaMKII is dephosphorylated primarily by PP2A, whereas in the PSD, dephosphorylation is almost exclusively mediated by PP1 (REF. 80). Remarkably, PP2A cannot dephosphorylate CaMKII in the PSD, even if it is added to the preparation[81]. The ability of PP1 to dephosphorylate CaMKII seems to depend on the fact that PP1 is immobilized in the PSD by scaffold proteins that include spinophilin, neurabin, yotiao and intermediate filaments[82]. However, it remains unclear which of these proteins interacts with the PP1 that is involved in CaMKII dephosphorylation. Taking into account the volume of the PSD, the estimated concentration of Thr286 sites is ~100 μM, significantly higher that the K_m of PP1 (1–10 μM). It is therefore likely that the PSD PP1 is saturated when CaMKII becomes hyperphosphorylated. These properties of PSD PP1 have been incorporated into the latest switch models.

The revised switch models also take into consideration the finding that CaMKII autophosphorylation requires Ca^{2+}/calmodulin to expose the Thr286 site. In the revised model, the basal level of Ca^{2+}/calmodulin, although low, can drive phosphorylation, consistent with experimental results[14]. So, the perpetuation of the highly phosphorylated 'on' state can be visualized as follows. Initially, all six Thr286 sites within a ring of subunits are phosphorylated. PP1 dephosphorylates a subunit at random. When this dephosphorylated subunit

binds a single molecule of Ca^{2+}/calmodulin at the basal Ca^{2+} concentration, the Thr286 site on this subunit will be rephosphorylated by its phosphorylated neighbour. This example serves to illustrate the importance of the ring structure for the perpetuation of the 'on' state: any subunit that becomes dephosphorylated will have a neighbour to rephosphorylate it. This condition would not be met by a linear array of subunits.

To investigate how the kinase and phosphatase reactions interact, mathematical analysis and computer simulations have been used. The results show that a group of kinase molecules in the PSD can exist in two stable states at basal Ca^{2+} level. A computer simulation of these stochastic reactions (see Supplementary Information online) provides an insight into how bistability arises. Switch function depends crucially on the creation of a special chemical environment within the PSD. For instance, bistability would not occur if PP2A could diffuse into the PSD and dephosphorylate CaMKII. Bistability also requires that PP1 activity in the PSD be quantitatively low. This makes sense because large amounts of energy would otherwise have to be used to rephosphorylate sites that are constantly being dephosphorylated by PP1. The low reaction rate within the PSD creates a nearly 'frozen' state that is energy efficient. As we discuss below, recent experimental results provide evidence that PP1 activity in the PSD might indeed be very low.

A crucial aspect of information storage by a protein switch is that it must be able to store information despite protein turnover. In the case of CaMKII, turnover occurs in about one month[83]. The saturation of PP1 by the hyperphosphorylated CaMKII molecules in the PSD produces a novel form of communication between CaMKII holoenzymes that might be important in resisting the effects of turnover. Holoenzymes do not phosphorylate each other directly, but if an unphosphorylated holoenzyme were to replace a highly phosphorylated one, the new holoenzyme would be subject to lower phosphatase activity because nearby phosphorylated holoenzymes would saturate the phosphatase. The model therefore predicts that the newly inserted holoenzyme would be quickly phosphorylated, and that the original state of the switch would be restored. This theoretical work elucidates principles by which known properties of CaMKII and PP1 in the PSD could produce a protein switch that is capable of long-term information storage.

Persistent CaMKII activity and synaptic memory. We have already summarized data indicating that CaMKII is necessary and sufficient for LTP induction. But this evidence does not necessarily mean that CaMKII also functions as a memory molecule that is responsible for maintaining LTP. It is possible that CaMKII acts only as a trigger, and that other downstream events are responsible for the persistence of LTP and memory. In this section, we review experimental evidence that is relevant to this issue.

Mutation of Thr286 prevents persistent activation of the kinase and blocks LTP induction. This indicates that persistence is important; however, this mutation does not distinguish between short-term and long-term persistent activity (FIG. 3). It is therefore possible that the importance of autophosphorylation is simply to extend the period of CaMKII activity from seconds to minutes; a few minutes of CaMKII activity might be sufficient to trigger persistent downstream processes that are the actual molecular memory. However, this would not explain why CaMKII activity is persistent for at least one hour after LTP induction, and why there is persistent phosphorylation of CaMKII targets. Recent work[57] has shown that GluR1 is phosphorylated by CaMKII for at least one hour after LTP induction, and that GluR1 is dephosphorylated by depotentiation protocols (FIG. 4c). Further work[84] indicates that procedures that produce depotentiation reduce CaMKII phosphorylation at Thr286 (FIG. 4d). So, persistent CaMKII activity is required for maintaining GluR1 phosphorylation. It is difficult to imagine why such complex regulation should occur if it is unimportant.

The most crucial test of any hypothesis of synaptic memory is to induce a memory, interfere with a putative storage molecule, and see if the memory disappears. This approach has been used to test the role of CaMKII in LTP maintenance, yielding mixed results. In all studies but one[85], postsynaptic application of a CaMKII inhibitor after LTP induction did not block LTP maintenance, even though application of the same inhibitor before induction produced a complete block of LTP[26,28]. The inability to block LTP maintenance could be taken as support for the idea that the storage process has been transferred to a downstream process. But the result could also be explained if phosphatases at the PSD were too inactive to dephosphorylate CaMKII in the hour for which these experiments lasted. Indeed, theoretical estimates indicate that PP1 activity might be so low that it would take more than a day for CaMKII to be dephosphorylated. Importantly, recent work[86] provides experimental evidence for low phosphatase activity. A 15-minute application of H7, a broad-spectrum kinase inhibitor, led to reduced phosphorylation in several proteins, but had no effect on CaMKII phosphorylation at the PSD. Proving the role of CaMKII in LTP maintenance might require methods that allow sufficient time for the low activity of PP1 to have an effect.

CaMKII- and translation-dependent processes. According to a prominent model, protein kinases are important only in the early phase of LTP, whereas the late phase depends on protein synthesis[87]. This might explain why protein-synthesis inhibitors selectively reduce the late phase of LTP. Early experiments showed that the early processes could be bypassed by stimulation of the cyclic AMP pathway, leading to potentiation through activation of protein kinase A (PKA) and the cAMP-responsive-element-binding protein (CREB)[88,89]. However, recent work has shown that NMDA antagonists or CaMKII inhibitors can block the potentiation and the structural changes that are produced by activation of the cAMP pathway[90,91]. One likely possibility is that cAMP inhibits PP1, leading to CaMKII activation[92]. In any case, there is no definitive evidence that cAMP can strengthen synaptic transmission by a CaMKII-independent mechanism. This agrees with the finding

that pharmacological or genetic interference with CaMKII blocks both early and late phases of LTP, although not in very young animals[93].

There is a growing knowledge of how the dendritic synthesis of proteins, including CaMKII, is affected by synaptic activity. In the hippocampus, α-CaMKII messenger RNA is distributed in dendrites and cell bodies, whereas β-CaMKII mRNA is present only in cell bodies[94,95]. Targeting of α-CaMKII mRNA requires several *CIS*-ACTING ELEMENTS in the 3′ untranslated region of the mRNA, one of which depends on synaptic activity for dendritic targeting[96,97]. Neuronal depolarization recruits α-CaMKII mRNA into granules that are targeted to dendritic processes[98]. Dendrites have the machinery necessary for translation, and local protein synthesis might participate in synaptic plasticity (see REF. 99 for review). For example, exposure to brain-derived neurotrophic factor stimulates local protein synthesis of α-CaMKII mRNA in isolated dendrites, and this local synthesis is necessary to induce changes in synaptic efficacy[100]. As indicated earlier, induction of LTP in hippocampal slices increases not only phosphorylation, but also the level of α-CaMKII, indicating that rapid translation of CaMKII mRNA also occurs in dendrites[21,101].

A structural component of LTP expression organized by CaMKII, as outlined in FIG. 7, could potentially explain the inhibition of late-phase LTP by protein-synthesis inhibitors and other agents that interfere with transcription[102]. It would not be surprising if the addition of new molecules to the synapse depleted local precursor pools and required the synthesis of additional proteins[99]. If protein synthesis were blocked, the depletion of available pools would eventually lead to a decrease in transmission (that is, to a decrease in late-phase LTP). According to this view, CaMKII acts as a synaptic tag[103] that can store information even if protein-synthesis-dependent expression mechanisms fail. Indeed, there is strong behavioural evidence (reviewed in REF. 104) that amnesia produced by the brief application of protein-synthesis inhibitors can be reversible — memory recovers over time — as would be expected if expression, rather than storage, were affected.

CaMKII in synaptic plasticity *in vivo*

The hippocampal slice has been an excellent model by which to elucidate the role of CaMKII in LTP, but the question remains as to whether this form of plasticity occurs *in vivo*, and whether it is used in brain regions other than the hippocampus. Here, we will consider several cases in which these issues have been explored.

CaMKII in the retinotectal system. In the frog, retinal neurons directly innervate the tectum and form a retinotopic map that develops gradually. In this system, it has been possible to monitor the development of axonal branching, dendritic arborization and synaptic connections, and to study how constitutively active CaMKII affects these processes *in vivo*. During early development, retinotectal synapses are primarily silent, lacking AMPA receptors. At this time, CaMKII is expressed at low levels[105]. Viral infection *in vivo* permits expression of a truncated fragment of CaMKII that is constitutively active (tCaMKII) in tectal neurons, without affecting their presynaptic partners, the retinal axons. This treatment increases Ca^{2+}-independent CaMKII activity by about 30% (REF. 106). As a result, there is a profound change in the property of retinotectal synapses: the strength of AMPA-receptor-mediated transmission in increased and the fraction of silent synapses decreases[61] (FIG. 8). This is what would be predicted by *in vitro* properties of hippocampal LTP.

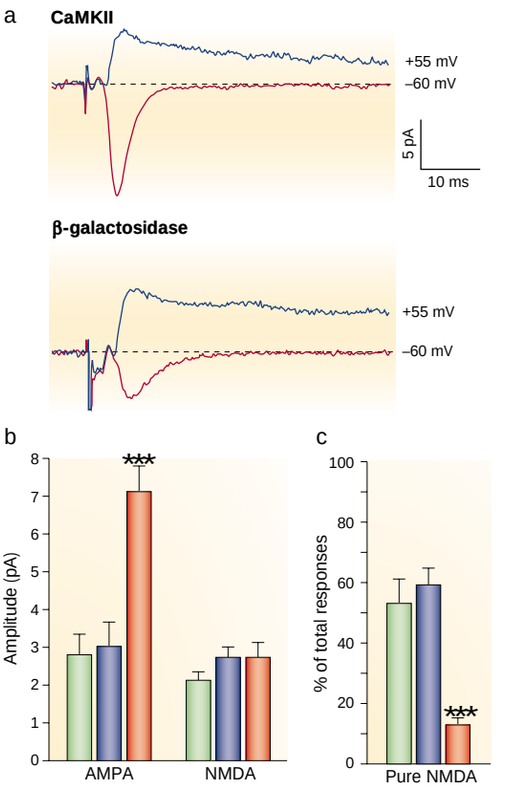

Figure 8 | **CaMKII activity promotes synaptic maturation *in vivo*. a** | Expression of constitutively active calcium/calmodulin-dependent protein kinase II (CaMKII), but not β-galactosidase, in frog optic tectal neurons increases the strength of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-receptor-mediated transmission in retinotectal synapses without altering NMDA (*N*-methyl-D-aspartate)-receptor-mediated responses. During normal development, the AMPA/NMDA receptor ratio in retinotectal synaptic responses increases owing to a selective increase in the AMPA-receptor-mediated component. Expression of CaMKII mimics normal synaptic maturation. Recordings at a holding potential of –60 mV (red) show the AMPA-receptor-mediated current. Recordings at +40 mV (blue traces) show both AMPA- (early) and NMDA-receptor-mediated (late) synaptic components. **b** | Active CaMKII (red bars) increases the AMPA-, but not the NMDA-receptor-mediated component of the synaptic response compared with uninfected controls (green bars) or with controls infected with β-galactosidase (blue bars). **c** | Active CaMKII decreases the fraction of silent synapses defined as having no AMPA-receptor-mediated synaptic component. ***$P < 0.01$. Adapted with permission from REF. 61 © 1996 American Association for the Advancement of Science.

CIS-ACTING ELEMENT
A regulatory genetic element that is located in the same DNA molecule as the gene that is being regulated.

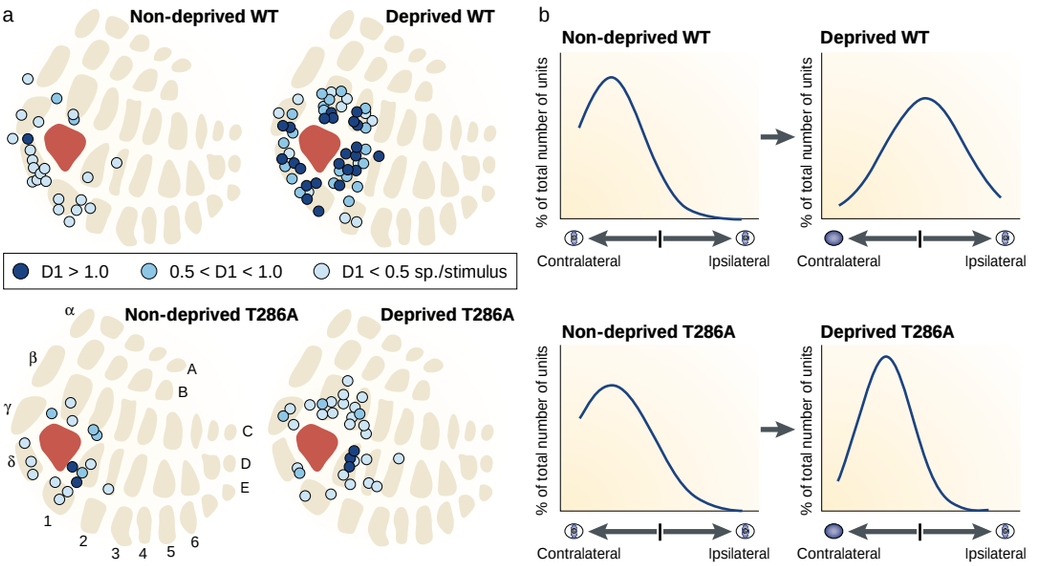

Figure 9 | **CaMKII affects cortical plasticity. a** | Expression of the T286A mutant of α-calcium/calmodulin-dependent protein kinase II (α-CaMKII^{T286A}) depresses plasticity in the somatosensory cortex induced by removing all but one whisker. Barrels in the somatosensory cortex are shown in beige. Letters and numbers label the whiskers and the barrels to which they project; each whisker projects mainly to a single barrel and weakly to surrounding barrels. The connections of the D1 whisker to the surrounding barrels in wild-type (WT) animals (upper left) are weak — less than 1 spike per stimulus (light-blue circles). Removal of all but the D1 whisker changes the strength of its connections to neighbouring barrels such that neurons in nearby barrels become more strongly responsive to the D1 whisker. Most neurons fire between 0.5 and 1 spikes per stimulus (blue circles) or more than 1 spike per stimulus (dark-blue circles). The strength of inputs from the D1 whisker to neighbouring barrels is comparable in wild-type and mutant animals. However, the α-CaMKII^{T286A} animals do not show the deprivation-induced plasticity of wild-type animals. **b** | Expression of α-CaMKII^{T286A} impairs ocular-dominance plasticity produced by monocular deprivation in mice. Most cells in wild-type or transgenic mice respond preferentially to visual stimulation of the contralateral eye. After deprivation of the contralateral eye in wild-type mice, visual cortical neurons become more responsive to the open ipsilateral eye. This is seen as a rightward shift in the curve in the upper right panel. In α-CaMKII^{T286A} animals, visual deprivation fails to induce ocular-dominance plasticity, so that the response curve in deprived transgenic mice is similar to that of non-deprived animals[113]. Part **a** adapted with permission from REF. 111 © 2000 Macmillan Magazines Ltd.

Experiments in the retinotectal system have shown that CaMKII expression affects the structural plasticity of dendrites and axons. By labelling presynaptic retinal axons or the dendrites of tectal cells with fluorescent markers, it is possible to visualize their structure *in vivo* and to follow their development. Before the onset of CaMKII expression, the growth rate of dendritic arborizations is rapid, and fine dendritic branches are constantly added and retracted. NMDA receptor antagonists block both the rapid branch dynamics and the elaboration of the dendritic arborization[105]. The growth and branch dynamics of dendritic arborizations slow down once the arborization becomes complex. This transition to a slower growth rate correlates with the time of CaMKII expression[107]. To test whether CaMKII expression is responsible for the slower growth rate, tCaMKII was expressed before this transition normally occurs. This led to premature stabilization of the dendritic arborization by decreasing the rates of branch addition and retraction. Conversely, inhibiting endogenous CaMKII in tectal cells by the expression of an inhibitory peptide increased both dendritic arborization branching and branch dynamics[108]. Retinal axon structure is also dynamic, showing rapid branch additions and retractions[109]. Mirroring the stabilization of the dendritic arborization, the structural plasticity of retinal axons decreased after viral expression of tCaMKII in tectal neurons[106]. This implies that a signal from tectal cells stabilizes axonal structure.

These findings indicate that axonal and dendritic structure is dynamic, but can be stabilized by a CaMKII-dependent process. Initially, the AMPA/NMDA receptor ratio is low and transmission is weak. Under these conditions, dendrites and axons rapidly add and retract branches, a dynamic behaviour that is regulated by visual system activity and NMDA receptor antagonists[105,109]. The newly formed dendritic branches might form synapses with newly added axonal branches. If NMDA receptors at these synapses detect correlated pre- and postsynaptic activity, they are strengthened by the CaMKII-dependent addition of AMPA receptors, leading to the stabilization of dendritic and axonal branches. If correlated activity does not occur, synaptic contacts might only be transient and local axon and dendritic dynamics continue. So, although synaptic and structural plasticity are often viewed as unrelated, they seem to be part of a common process that involves a CaMKII-dependent enhancement of synaptic strength.

CaMKII and cortical plasticity. In the primary somatosensory cortex of mice, the whiskers of the snout are mapped onto morphologically distinct arrangements of neurons termed BARRELS. Neurons within layer 4 of each barrel receive excitatory input that is driven

BARRELS
Cylindrical columns of neurons that are seen in the rodent somatosensory neocortex. Each barrel receives sensory input from a principal whisker follicle, and the topographical organization of the barrels corresponds precisely to the arrangement of whisker follicles on the face.

primarily by one whisker, with moderate input from neighbouring whiskers. Within each barrel, neurons in layers 2/3 are also driven predominantly by the same whisker. Removal of all but one of the whiskers results in expansion of the cortical area driven by the remaining whisker: its stimulation now excites cortical neurons of layers 2/3, not only in the same barrel field, but also in neighbouring barrels. Plasticity in layers 2/3 of this system extends into adulthood. Glazewski and colleagues[110,111] examined how this form of synaptic plasticity is affected by deletion of α-CaMKII or by knocking-in a form of α-CaMKII that cannot be phosphorylated at Thr286 (α-CaMKII^{T286A})[31]. In both cases, animals failed to show cortical plasticity (FIG. 9a). As they also failed to show cortical LTP[112], a reasonable interpretation of these data is that CaMKII-dependent LTP is required for cells in layer 4 to form strong connections with layer 2/3 in the neighbouring barrels.

Experiments in the visual cortex show similar effects of the T286A mutation on a different form of experience-dependent plasticity. During the so-called 'critical period' of visual development, occluding vision in one eye can modify the degree to which cortical cells are driven by the two eyes. In normal mice, cortical cells tend to be driven most strongly by the contralateral eye. However, after a period of monocular deprivation, the influence of the non-deprived ipsilateral eye is greatly increased. This form of plasticity is greatly reduced by α-CaMKII^{T286A} (REF. 113; FIG. 9b).

The role of CaMKII has now been examined in some of the main models that are used to study plasticity in the developing and adult nervous systems *in vivo*. The emerging picture is that deletion of α-CaMKII or interference with its persistent activation blocks both activity- and experience-dependent forms of synaptic plasticity.

CaMKII in behavioural memory

The first study of the role of CaMKII in learning and memory was made in α-CaMKII knockout mice. These mice were deficient in LTP and hippocampus-dependent spatial learning tasks[114,115]. However, interpretation of these results was complicated by the fact that the mice also suffered epileptic seizures, which might affect brain development and learning. Furthermore, the link between hippocampal LTP and learning could not be convincingly made in these animals because they still showed some hippocampal LTP[30]. Memories are thought to be stored initially in the hippocampus, and then consolidated in the cortex over several weeks. Interestingly, α-CaMKII heterozygous animals, which express only half of the wild-type protein level, show normal hippocampal LTP, but no cortical LTP. In behavioural tasks, these animals learn normally, but subsequently forget, presumably because the normal transfer of information from hippocampus to cortex is not possible[112].

With the development of the KNOCK-IN CaMKII^{T286A} mouse[31], it became possible to test whether the autophosphorylation of CaMKII and the resulting persistent activity are required for learning. These animals showed no hippocampal LTP or LTD with a variety of stimulation protocols. As shown in FIG. 10, they were strongly deficient in spatial memory tasks. Furthermore, hippocampal PLACE CELLS, which form a spatial map of the environment and are thought to be required for learning spatial tasks, are unstable in α-CaMKII^{T286A} mice[31,116]. These data are consistent with a requirement for autophosphorylation of α-CaMKII at Thr286 for the ability of mice to learn and remember hippocampus-dependent tasks.

Mayford and colleagues used a different strategy to test the role of the switch from Ca^{2+}-dependent to Ca^{2+}-independent CaMKII activity in learning and memory. They generated transgenic mice in which Thr286 was replaced with aspartate, a substitution that mimics the effect of autophosphorylation, significantly increasing Ca^{2+}-independent kinase activity *in vitro*[117,118]. The α-CaMKII^{T286D} transgene was driven by the α-CaMKII promoter, leading to lines of mice that expressed the transgene primarily within the forebrain, hippocampus, amygdala and striatum. Mice with strong transgene

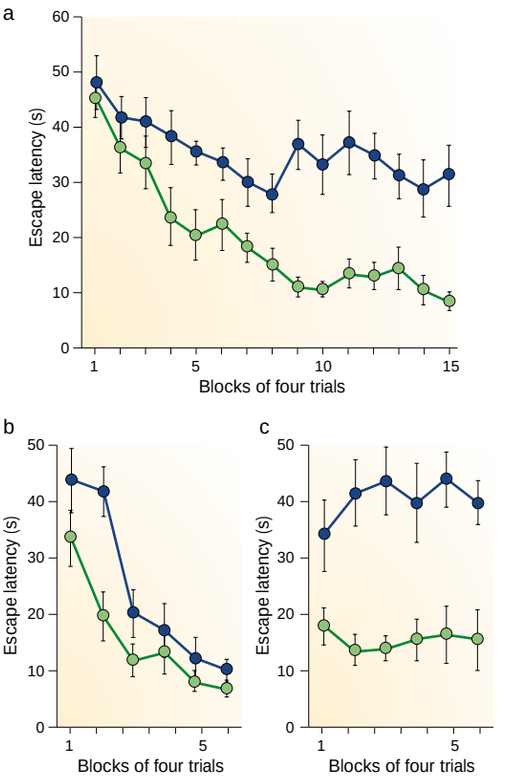

Figure 10 | **Learning and memory are affected in animals that express a mutant form of CaMKII. a** | Wild-type animals (green) learn to find a hidden platform in the MORRIS WATER MAZE, whereas animals that express the T286A mutant of α-calcium/calmodulin-dependent protein kinase II (α-CaMKII^{T286A}) show limited learning (blue). **b** | Wild-type and mutant animals perform similarly when the platform is visible. **c** | When the visible platform is hidden, wild-type animals that have just learned the task can immediately find the platform location. α-CaMKII^{T286A} animals cannot locate the platform, indicating that memory is impaired[31]. Adapted with permission from REF. 31 © 1998 American Association for the Advancement of Science.

GENE KNOCK-IN
The insertion of a mutant gene at the exact site of the genome at which the corresponding wild-type gene is located. This approach is used to ensure that the mutant gene is regulated in the same way as the endogenous locus.

PLACE CELLS
Hippocampal principal cells that fire selectively when an animal is in a particular location in its environment, presumably encoding a spatial map of its surroundings.

MORRIS WATER MAZE
A learning task in which an animal is placed in a pool filled with opaque water and has to learn to escape to a hidden platform that is placed at a constant position. The animal must learn to use distal cues, and the spatial relationship between them and the platform. Learning in this task involves the hippocampus.

TETRACYCLINE-DEPENDENT TRANSACTIVATOR SYSTEM
A system that allows the temporal control of gene expression in eukaryotic systems through the administration of tetracycline. It is based on two key elements: the tetracycline-dependent transactivator protein (tTA) and the target gene under the control of a tTA-responsive element. When these elements are transfected into eukaryotic cells, the tTA binds to the tTA-responsive element to initiate transcription. Tetracycline can then be administered to stop expression of the target gene.

expression had about twice the amount of Ca^{2+}-independent kinase activity seen in wild-type mice. The transgenic animals failed to show LTP in response to relatively weak stimuli (in the 10-Hz range)[119]. This is significant because exploratory behaviours that are associated with learning hippocampus-dependent spatial tasks are accompanied by hippocampal activity in this frequency range, the so-called theta frequency. Indeed, the mice failed to learn hippocampus-dependent spatial tasks that depended on visual[120] or olfactory cues[121]. This inability might be related to the lack of stable hippocampal place fields in these animals[122]. One aspect of these studies remains puzzling: although the LTP produced by 10-Hz stimulation is blocked, as would be predicted by the ability of active CaMKII to occlude LTP (FIG. 6), some LTP can still be induced if stronger stimulation is used. One explanation of this residual LTP is that the T286D mutation causes premature binding to the NMDA receptor, thereby preventing some of the kinase from diffusing to synapses.

Spatial and temporal control of α-CaMKII^{T286D} expression was achieved by crossing α-CaMKII^{T286D} transgenic animals to other transgenic mice that expressed a tetracycline transactivator driven by the CaMKII promoter[123]. This TETRACYCLINE-DEPENDENT TRANSACTIVATOR (TET-OFF) SYSTEM allowed the suppression of α-CaMKII^{T286D} expression when animals were given the tetracycline analogue doxycycline. In principle, these animals could make possible a crucial experiment: raising the mice with doxycycline so that the transgene is 'off' during development, and then turning α-CaMKII^{T286D} on either during or after training. This would determine whether CaMKII activity is required for learning and memory, and would rule out effects of altered CaMKII function during development. Unfortunately, prolonged exposure to doxycycline itself during development impairs memory.

However, the 'tet-off' system is suitable for asking whether constitutively active CaMKII can disrupt stored memories. This would be expected if the small fraction of synapses that is strengthened during learning became indistinguishable from the large fraction that was strengthened by the mutant kinase. To test this possibility, doxycycline was applied before learning[123] (to ensure that CaMKII was normal). After learning, the doxycycline was removed. The results showed that memory was now impaired, consistent with the idea that the expressed active CaMKII had 'overwritten' a stored memory.

Conclusions

Remarkable progress has been made in understanding the role of CaMKII in LTP. All of the key experiments have been replicated in several laboratories and using independent methods. There is thus little doubt that CaMKII is activated during LTP induction and that this activation is necessary and sufficient for LTP. It is also clear that CaMKII can strengthen synaptic transmission by multiple mechanisms. One of them involves the direct phosphorylation of AMPA receptors. Another involves the addition of AMPA receptors to synapses, which might depend on changes in receptor trafficking and on the organized addition of anchoring sites for AMPA receptors. The recently discovered binding of CaMKII to NMDA receptors in the PSD could be an initial step in this structural process. It is clear that characterizing the multiple binding interactions and obtaining a clear structural model of the PSD will be crucial in understanding the mechanisms by which CaMKII increases synaptic strength.

The demonstration that CaMKII activity persists for at least one hour after LTP induction, and that LTP is blocked by a mutation that prevents persistent activation, indicates that CaMKII functions as more than just a trigger during LTP induction. Computational modelling of kinase–phosphatase interactions within the PSD indicates that the kinase could serve as a molecular basis of long-term synaptic memory. Indeed, the ring structure of the kinase, its extensive phosphorylation, the exclusion of PP2A from the PSD and the presence of PP1 can be seen as an integrated solution to the design of an energy-efficient and stable switch that is capable of long-term information storage. However, it is also possible that only short-term persistent activation of CaMKII is required; this might trigger a downstream process that constitutes true long-term synaptic memory. Further experiments will be required to distinguish between these possibilities.

Although CaMKII clearly has an important role in LTP, the role of LTP itself in normal brain plasticity processes has not been proven unequivocally. Thus, an important line of research has been to determine whether the CaMKII mutations that interfere with LTP also interfere with natural forms of plasticity. The results of this work have been positive: interfering with CaMKII activation disrupts developmental changes in synaptic function, experience-dependent plasticity and behavioural memory. An important aspect of this work has been the fact that CaMKII affects *in vivo* plasticity in different brain regions in a way that is predicted by the study of LTP at CA1 hippocampal synapses. These results indicate that CaMKII-dependent processes that are involved in hippocampal LTP are quite general. Indeed, invertebrates have a homologous kinase that has a key role in synaptic function[124] and learning[125]. It is therefore probable that CaMKII is of early evolutionary origin and has long been specialized for information storage.

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DATABASES

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