or derivatives was cotransfected with 0.25 μg of a copia-lacZ internal control and 1 μg of a reporter construct containing two C/EBP binding sites upstream of a minimal thymidine kinase promoter and the gene encoding luciferase. After 48 hours, luciferase and β-galactosidase activities were measured. Fold transactivation relative to no transactivator is listed as the average of three experiments (Figs. 2 through 5). All DmC/EBP derivatives, C/EBP-α, and C/EBP-δ were of the expected size and expressed in amounts similar to that of DmC/EBP, as judged by protein immunoblot with antibodies to DmC/EBP (*6*), C/EBP-α [L. M. Scott, C. I. Civin, P. Rørth, A. D. Friedman, *Blood* **80**, 1725 (1992)], and C/EBP-δ.

10. H.-P. Gerber *et al.*, *Science* **263**, 808 (1994).
11. T. E. Ellenberger, C. J. Brandl, K. Struhl, S. C. Harrison, *Cell* **71**, 1223 (1992); P. König and T. J. Richmond, *J. Mol. Biol.* **233**, 139 (1993).
12. J. Kim, D. Tzamarias, T. Ellenberger, S. C. Harrison, K. Struhl, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4513 (1993); P. F. Johnson, *Mol. Cell. Biol.* **13**, 6919 (1993); M. Suckow, V. von Wilcken-Bergmann, B. Muller-Hill, *EMBO J.* **12**, 1193 (1993).
13. Theoretically, an additional function might not be severely affected by any of the single amino acid substitutions or might not be affected independently of DNA binding.
14. D. Ron and J. F. Habener, *Genes Dev.* **6**, 439 (1992); P. Descombes and U. Schibler, *Cell* **67**, 569 (1991).
15. M. Vallejo, D. Ron, C. P. Miller, J. F. Habener, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4679 (1993).
16. K. P. LeClair, M. A. Blanar, P. A. Sharp, *ibid.* **89**, 8145 (1992); B. Stein, P. C. Cogswell, A. S. Baldwin Jr., *Mol. Cell. Biol.* **13**, 3964 (1993).
17. M. Wegner, Z. Cao, M. G. Rosenfeld, *Science* **256**, 370 (1992); C. Nerlov and E. B. Ziff, *Genes Dev.* **8**, 350 (1994).
18. W. Driever, J. Ma, C. Nüsslein-Volhard, M. Ptashne, *Nature* **342**, 149 (1989).
19. J. Malicki, K. Schughart, W. McGinnis, *Cell* **63**, 961 (1990); N. McGinnis, M. A. Kuziora, W. McGinnis, *ibid.*, p. 969; J. J. Zhao, R. A. Lazzarini, L. Pick, *Genes Dev.* **7**, 343 (1993).
20. X. Li and M. Noll, *Nature* **367**, 83 (1994).
21. R. Steward, *Science* **238**, 692 (1987); C. Rauskolb, M. Peifer, E. Wieschaus, *Cell* **74**, 1101 (1993).
22. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; G, Gly; H, His; I, Ile; K, Lys; L, Leu; N, Asn; P, Pro; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.
23. Pw15 was digested with Bgl II and partially digested with Mlu I, and the oligonucleotide 5′-(CGCG)AAAATGAGATCTTAGGTACCGCGGCCGCTCTAGAG(GATC)-3′ was inserted.
24. Activation domains were cloned by PCR (confirmed by sequencing), with the following primers and complementary DNA or genomic DNA as template. N-bZIP: 5′-CCGGATCCATG**GCGGCCGC**AGGAGC-3′; VP-16: 5′-CGGGATCCATGGGCGAGGACGTG-GCGAT-3′ and 5′-TTGAA**GCGGCCGC**CAGGC-CCACCGTACTCGTC-3′; GAL4: 5′-TTGAA**GCG-GCCGC**CAGGCTCTTTTTTTGGGTTTGG-3′ and $GAL4_{short}$: 5′-CGGGATCCATGATCACTACAGG-GATGTTT-3′, $GAL4_{long}$: 5′-CGGGATCCATGGC-CAATTTTAATCAAAGTGG-3′; C/EBP-α_{1-257}: 5′-CGGGATCCATGGAGTCGGCCGAC-3′ and 5′-TT-GAA**GCG GCC GC**GGGGTGCGGACCAGC-3′; C/EBP-δ_{1-164}: 5′-CGGGATCCATGAGCGCCGC-GCTT-3′ and 5′-TTGAA**GCGGCCGC**AGGAGGCT-CCGGCGA-3′. Deletion of internal Not I fragments created C/EBPδ_{1-144} and C/EBP-α_{1-153}; for C/EBP-α_{1-153}, the Not I sites were filled in. Bam HI–Not I fragments were cloned into a vector with Bam HI 5′ of DmC/EBPs ATG (*6*), with the use of the internal Not I site. The sequence surrounding this site has been corrected to LPHLAAAAGAHNLLK (*22*) on the basis of new DNA sequencing. Mutagenesis with 5′-CCTG**GCGGCCGC**·GCAGGAGCA-3′ recreated the corrected reading frame in affected fusions.
25. P. Rørth, thesis, University of Copenhagen, Denmark (1993).
26. The Xho I site was introduced by mutagenesis of DmC/EBP with 5′-CCTCCACCTCGAGGGAGCG-CAC-3′. The GCN4 zipper was inserted as an Xho I–Xba I fragment generated by PCR with the oligonucleotides 5′-GCCTCGAGCTTGAAGACAAGGTT-GAA-3′ AND 5′-GGTCTAGATCAGCGTTCGCCA-ACTAA-3′. The K_d for DNA binding of DmC/EBP-GCN4zip was slightly elevated, which may explain the decreased rescue.
27. I thank P. Beachy for Schneider cells, copia-lacZ, and an Actin5C plasmid and S. L. McKnight for the C/EBP-δ antibody. I am grateful to J. Shuman, C. Thompson, A. Fire, A. Spradling, and S. L. McKnight for discussions and helpful comments on the manuscript. Supported by fellowships from the Danish Medical Research Council and the Danish Cancer Society and by the Carnegie Institution of Washington.

20 July 1994; accepted 18 October 1994

Potentiated Transmission and Prevention of Further LTP by Increased CaMKII Activity in Postsynaptic Hippocampal Slice Neurons

D. L. Pettit, S. Perlman, R. Malinow*

Calcium-calmodulin–dependent protein kinase II (CaMKII) is a necessary component of the cellular machinery underlying learning and memory. Here, a constitutively active form of this enzyme, CaMKII(1–290), was introduced into neurons of hippocampal slices with a recombinant vaccinia virus to test the hypothesis that increased postsynaptic activity of this enzyme is sufficient to produce long-term synaptic potentiation (LTP), a prominent cellular model of learning and memory. Postsynaptic expression of CaMKII(1–290) increased CaMKII activity, enhanced synaptic transmission, and prevented more potentiation by an LTP-inducing protocol. These results, together with previous studies, suggest that postsynaptic CaMKII activity is necessary and sufficient to generate LTP.

CaMKII mediates numerous physiological processes triggered by a rise in intracellular Ca^{2+} ions (*1*). In the brain, its abundance at excitatory synaptic contacts (*2*) makes it strategically placed to respond to localized Ca^{2+} transients. Its molecular properties suggest that this enzyme could convert a transient signal to a long-lasting modification (*3*). Particular attention has been paid to the role of CaMKII in LTP, a form of activity-dependent synaptic plasticity that may underlie some forms of learning. LTP is triggered by a conditioning protocol that transiently activates postsynaptic *N*-methyl-D-aspartate (NMDA) receptors (*4*) and produces an increase in postsynaptic Ca^{2+} concentration (*5*). The biochemical steps occurring after this increase in Ca^{2+} have not been established, although several protein kinases have been implicated (*6–9*). In particular, inhibition of CaMKII activity in neurons, either with peptide inhibitors (*10*) or genetically (*11*), prevents LTP.

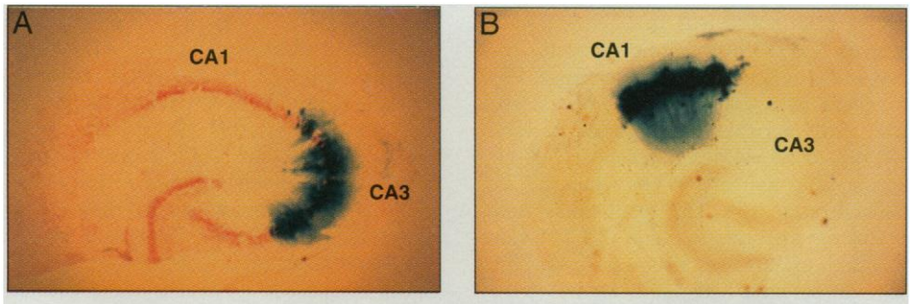
To test the hypothesis whether postsynaptic CaMKII activity is sufficient to generate LTP, we used vaccinia virus (VV) infection to introduce recombinant products into neurons of hippocampal slices. Slices were prepared from 9- to 13-day-old rats by standard methods (*12*). Under visual guidance, the extracellular space of the CA1 pyramidal cell body layer was injected with a solution containing purified VV (*13*). Slices were then incubated at 35°C for 4 to 16 hours to allow for infection and expression of the recombinant gene products. Initially, experiments with a VV encoding β-galactosidase (BGVV) were performed to test for neuronal expression of recombinant virus product. Infected slices were fixed, stained with X-gal, and cleared (Fig. 1, A, B, and C). Within 4 hours of infection, recombinant expression could be detected (*14*). Overlapping injections, carefully controlled to ensure maximal delivery of virus-rich solution to the tissue, produced continuous staining of the injected area (Fig. 1, A and B). Thin sections (40 μm) through slices indicate that such injections can infect 100% of cells in the injected area (Fig. 1A). Injections can be restricted to targeted regions, for example, the postsynaptic CA1 region (Fig. 1B). Recombinant products in such infections are not expressed in presynaptic structures because to express recombinant products VV requires translational machinery not found in presynaptic axons or terminals (*15*). The cell bodies of the presynaptic neurons show no expression of recombinant products in infections of the CA1 region (Fig. 1B). Electrophysiological properties of cells infected with BGVV were indistinguishable 6 hours after infections compared with those of uninfected cells (input resistance: 290 ± 3 megohms for cells from BGVV-infected slices, $n = 22$; 276 ± 1 megohms for uninfected cells, $n = 8$, $P > 0.5$, t test; resting membrane potential: 54 ±

D. L. Pettit and R. Malinow, Neuroscience Program, University of Iowa, Iowa City, IA 52242, USA, and Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.
S. Perlman, Departments of Pediatrics and Microbiology, Neuroscience Program, University of Iowa, Iowa City, IA 52242, USA.
*To whom correspondence should be addressed at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

0.8 mV for cells from BGVV-infected slices, $n = 28$; 54 ± 1 mV for uninfected cells, $n = 13$, $P > 0.5$, t test). In some experiments, recordings from infected cells were confirmed by inclusion of the β-galactosidase substrate CMFDG (Fig. 1D) in the recording pipette.

We designed a recombinant VV to produce a constitutively active form of CaMKII (*16*). We expressed amino acids 1 to 290 of α-CaMKII [CaMKII(1–290)], corresponding to its catalytic domain (*17*). This protein shows constitutive CaMKII activity (*17*, *18*), with no observed effects on other signal transduction pathways (*18*). To confirm that the virus [CaMKII(1–290)VV] made a functional product, we measured CaMKII activity in a cell line cotransfection assay system. GH3 pituitary cells were transfected by electroporation with a luciferase reporter plasmid under control of a rat prolactin promoter (2.5 kb). In this assay (*18*), CaMKII phosphorylates transactivators of the prolactin promoter resulting in an increase in luciferase production. Cells infected with CaMKII(1–290)VV showed increased luciferase activity compared with cells infected with BGVV (Fig. 2A). To determine the effects of these viruses on CaMKII activity in hippocampal slices, we infected slices with either CaMKII(1–290)VV or BGVV and allowed them to incubate for 6 hours. The infected CA1 region was dissected, and homogenates were tested for constitutive (Ca^{2+}-independent) CaMKII activity, constitutive protein kinase A (PKA) activity, or constitutive protein kinase C (PKC) activity (*19*). Slices infected with CaMKII(1–290)VV showed an increase of 14% in constitutive CaMKII activity compared with BGVV-infected controls ($n = 7$, $P < 0.025$, paired t test; Fig. 2, B and C). This increase is comparable with activity levels that have been measured during LTP (*20*). Constitutive PKA and PKC activities were not significantly changed in CaMKII(1–290)VV-infected slices compared with BGVV-infected slices ($P > 0.3$, $n = 6$ for each enzyme assay).

We then measured the effects of CaMKII(1–290)VV on synaptic transmission 4 to 8 hours after infection. At this time, whole-cell recordings were obtained from neurons in the regions injected with CaMKII(1–290)VV or BGVV. In preliminary experiments, increasing stimulus strength from a value yielding no transmission to a value just producing transmission (*21*, *22*) gave larger transmission in slices infected with CaMKII(1–290)VV (9.3 ± 2.3 pA, $n = 8$) compared with slices infected with BGVV (5.5 ± 3 pA, $n = 4$). These results from minimal stimulation suggested that transmission per fiber was greater in slices infected with CaMKII(1–290)VV. To test this more rigorously, we randomized the two viruses, and experiments were conducted and the data analyzed in a blind manner. There were no significant differences between the two groups with respect to resting membrane potential [CaMKII(1–290)VV-infected: −53 ± 0.81 mV, $n = 24$; BGVV-infected: −53 ± 0.83 mV, $n = 28$; $P > 0.5$] or input resistance [CaMKII(1–290)VV-infected: 266 ± 24 megohms, $n = 20$; BGVV-infected: 289 ± 30 megohms, $n = 22$; $P > 0.5$]. Synaptic transmission was elicited by a bipolar stimulating electrode placed outside the injected region. An input-output curve was obtained for each recording by measuring the average response at each of several



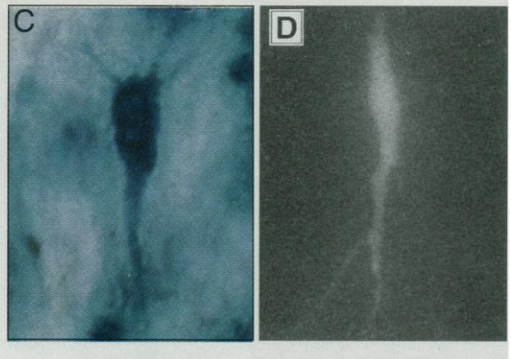
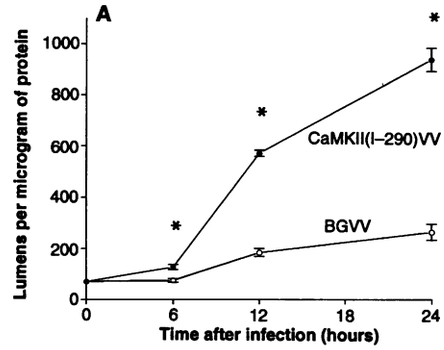
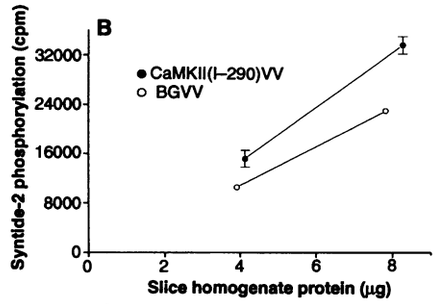


Fig. 1. Anatomically restricted expression of recombinant products with microapplication of vaccinia virus to hippocampal slices. (**A**) Photomicrograph of hippocampal slice injected with β-galactosidase–producing recombinant vaccinia virus (BGVV) into the extracellular CA3 cell body region. Infected slices were incubated (at 35°C for 8 hours) and fixed. Thin sections (40 μm) were prepared, stained with X-gal, and counter stained with neutral red (original magnification ×10). (**B**) Photomicrograph of hippocampal slice injected with BGVV into the extracellular CA1 cell body region, incubated, and stained with X-Gal (original magnification ×10). (**C**) High magnification (original magnification ×40) shows an individual CA1 pyramidal cell expressing β-galactosidase. (**D**) Fluorescence photomicrograph of a pyramidal cell filled with 200 μM CMFDG (Molecular Probes) by whole-cell pipette. CMFDG reacts with β-galactosidase to produce a fluorescent product.





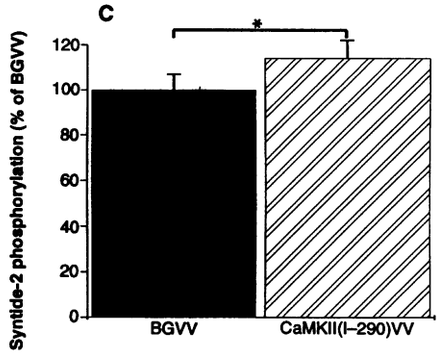


Fig. 2. Recombinant vaccinia virus makes functional CaMKII(1–290). (**A**) Lysates from GH3 cells infected with CaMKII(1–290)VV or BGVV (multiplicity of infection, 10:1) were assayed for CaMKII-dependent luciferase activity (*18*) at 0, 6, 12, and 24 hours after infection. (**B**) Phosphorylation of synthetic CaMKII-substrate peptide, syntide-2, by CA1 homogenates from slices infected with BGVV (○) or CaMKII(1–290)VV (●). Each point is the average (± SEM) of triplicates obtained for different amounts of homogenate protein. (**C**) Mean (± SEM) of syntide-2 phosphorylation as a percent of syntide-2 phosphorylation by BGVV-infected slices. The (*) indicates statistically significant difference of $P < 0.025$, $n = 7$, paired t test.

stimulus strengths (*23*). As more fibers were recruited by enhancing the stimulus strength, mean transmission increased more in the group injected with CaMKII(1–290)VV than in the group of slices injected with BGVV (Fig. 3B). The fourfold difference in transmission is unlikely to be due to differences in fiber excitability at the stimulation site because stimulating electrodes were placed well outside (>500 μm) the injected regions. Thus, these results are consistent with those obtained with minimal stimulation and indicate that the amount of transmission per fiber was greater in the group injected with CaMKII(1–290)VV.

Increased CaMKII activity could affect voltage-dependent ion channel function (*24*) and enhance neuronal excitability. If this occurred, spontaneous activity during the incubation period could generate conventional LTP, complicating interpretations. To address this possibility, we repeated the experiments described above in the presence of the NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV, 100 μM) during the preparation, virus injection, incubation, and recording period. Input-output curves for slices injected with either CaMKII(1–290)VV or BGVV were obtained and analyzed in a blind manner. Slices injected with CaMKII(1–290)VV showed a fourfold increase in transmission compared with slices injected with the control virus BGVV (Fig. 3C). Indeed, the results in the presence or absence of APV were indistinguishable. These results indicate that the enhanced transmission produced by increased CaMKII activity is not due to the generation of NMDA-dependent LTP during the incubation period.

We tested whether the enhanced transmission in slices infected with CaMKII(1–290)VV was related to LTP. Because LTP can show saturation (*25*), if increased postsynaptic CaMKII activity produces maximum LTP, no more LTP should be possible in cells infected with CaMKII(1–290)VV. This type of occlusion experiment has been used to test if two phenomena share common underlying mechanisms (*26*, *27*). We infected slices with either CaMKII(1–290)VV or BGVV and conducted and analyzed experiments in a blind manner. Whole-cell recordings were obtained from injected CA1 cell body regions. After a short baseline period (*28*), an LTP-inducing protocol pairing presynaptic activity with postsynaptic depolarization (see Fig. 4 legend) was delivered, and its effect on transmission was monitored for 15 to 60 min. The pairing protocol produced potentiation in the group of slices injected with BGVV (n = 13) (Fig. 4A). However, in the group of slices injected with CaMKII(1–290)VV, transmission returned to baseline levels within 3 to 5 min after the pairing protocol (n = 12) (Fig. 4B). This prevention of further LTP required new viral transcription because the effect was not observed if CaMKII(1–290)VV was injected into slices bathed in actinomycin D, an inhibitor of RNA synthesis (Fig. 4C). This result also argues against the possibility that co-injection of a compound generated during the production of CaMKII(1–290)VV was responsible for the effect on LTP. These findings support the hypothesis that increased CaMKII activity in postsynaptic cells triggers LTP maximally, preventing more LTP.

In this study we have used vaccinia virus infection to introduce recombinant products into selected neuronal populations in brain slices. The resulting expression is robust, can be restricted to selected populations of cells, and has no observed effect on synaptic transmission or plasticity. The virus is not transported transynaptically during the 4 to 16 hours of our experiments. This technique should be applicable to studies of any cloned protein and may prove useful as a means to rescue function in tissue from "knock-out" transgenic mice. This method of introducing proteins into neurons is preferable to delivery of proteins through a whole-cell pipette because it avoids the washout of cytosolic components necessary for cellular processes (for example, LTP).

We have generated a virus that produces constitutive CaMKII activity and used it to test whether postsynaptic expression of this protein mimics and prevents further LTP. The increase in constitutive CaMKII activity in slices induced by infection with CaMKII(1–290)VV is comparable with the increase seen after LTP induction (*20*, *29*). Neurons infected with CaMKII(1–290)VV showed increased responses to minimal stimulation as well as steeper input-output curves

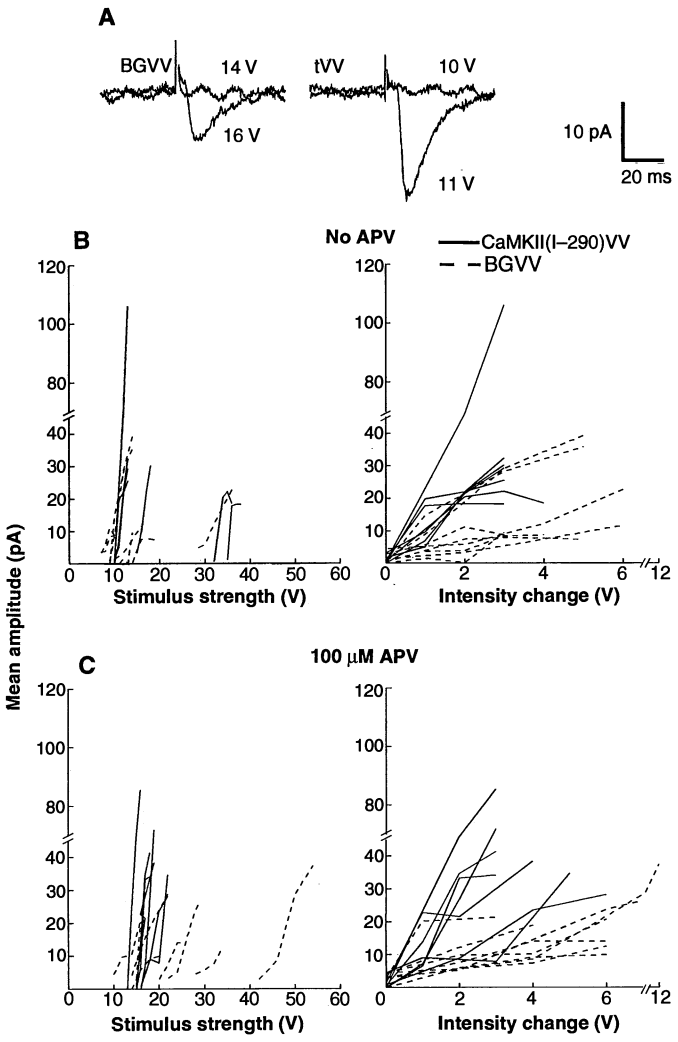


Fig. 3. Postsynaptic infection with virus encoding CaMKII(1–290) potentiates transmission. (**A**) Responses to subthreshold and minimal stimuli (stimulus voltage indicated) in slices infected with indicated virus (each trace is an average of 10 consecutive responses). (**B**) Mean elicited postsynaptic responses plotted as a function of the absolute stimulus strength (left) and change in intensity above a subthreshold stimulus (right). Each point is the average response to 10 stimuli evoked at 0.2 Hz. For each curve, a slope was calculated by linear regression. The slope of the input-output curve was greater in slices infected with CaMKII(1–290)VV (12.1 ± 4.1 pA/V, n = 7) than in slices infected with BGVV (3.4 ± 0.9 pA/V, n = 8; P < 0.01, Mann-Whitney U test). (**C**) Synaptic potentiation produced by postsynaptic CaMKII(1–290) is not prevented by APV. The experimental protocol is the same as in Fig. 3B, except that slices were prepared, incubated, and recordings obtained in the presence of 100 μM APV. The average slope of the curves was significantly greater in slices infected with CaMKII(1–290)VV (14.2 ± 3.6 pA/V; n = 7) compared with slices infected with BGVV (3.4 ± 0.9 pA/V, n = 8; P < 0.01, Mann-Whitney U test). In these experiments (A, B, and C) the CA1 region was infected with either BGVV or CaMKII(1–290)VV and incubated for 4 to 8 hours. Stimulus electrodes were placed in the stratum radiatum outside injected regions, and whole-cell recordings were obtained from CA1 neurons. The identity of the virus was not revealed until after all of the data had been collected and analyzed.

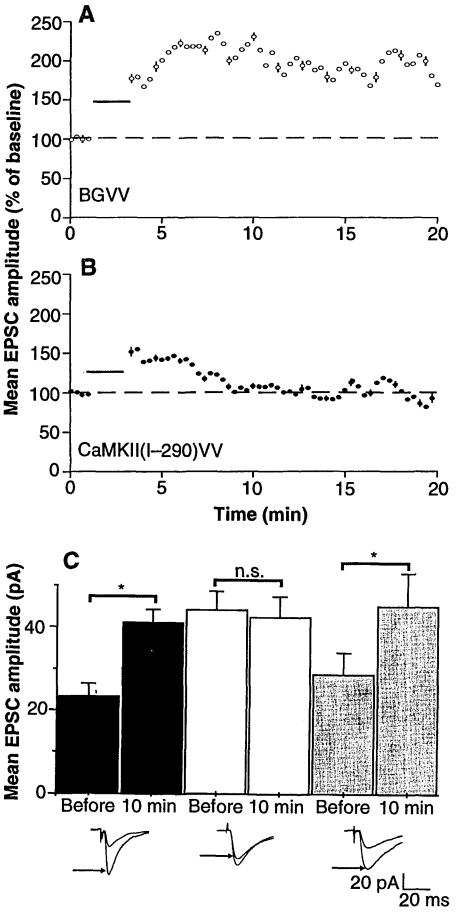


Fig. 4. Postsynaptic expression of CaMKII(1–290) prevents potentiation by LTP-inducing protocol. (**A** and **B**) Ensemble average amplitude of the excitatory postsynaptic current (EPSC) plotted as a function of time for transmission elicited 4 to 8 hours after slices were injected with either BGVV (A) (n = 13) or CaMKII(1–290)VV (B) (n = 12). CA1 regions of slices were infected with either BGVV or CaMKII(1–290)VV and incubated for 4 to 8 hours. The identity of the virus was not revealed until after all of the data had been collected and analyzed. Forty baseline EPSCs were recorded at a constant holding potential of −55 to −65 mV. A pairing protocol (the postsynaptic cell was depolarized to ~−15 mV while afferent stimuli were continued for 25 trials) was delivered (bar), and transmission was monitored at the original holding potential for 15 to 60 min. For each experiment, transmission is normalized to average response obtained before pairing. Ensemble averages are computed from these normalized data. Each point in the graph is a five-point average (± SEM) from ensemble averages. (**C**) Potentiation of transmission and prevention of LTP by postsynaptic infection with virus encoding CaMKII(1–290) are not observed in actinomycin D. The graph shows the mean (± SEM) of EPSC amplitude elicited before and 10 min after a pairing protocol in slices infected with BGVV (filled bars, n = 11), CaMKII(1–290)VV (white bars, n = 10), and CaMKII(1–290)VV in the presence of actinomycin D (10 μg/ml) (shaded bars, n = 7). In each experiment, stimulus strength was set to a level ~10% above a maximal stimulus that elicited no transmission. Note that in actinomycin D, slices infected with CaMKII(1–290)VV, transmission is not elevated before pairing and shows normal potentiation after pairing protocol. In all conditions larger responses were possible by increasing the stimulus strength, indicating that responses were not saturated (n.s.).

(*30*), supporting the hypothesis that postsynaptic CaMKII activity potentiates synaptic transmission.

Slices perfused with the NMDA receptor antagonist APV and infected with CaMKII(1–290)VV also had a fourfold synaptic enhancement, showing that the synaptic potentiation produced by CaMKII does not require NMDA activity and suggesting that CaMKII is part of the pathway leading to LTP downstream of the NMDA receptor. This result also suggests that increased postsynaptic CaMKII mediates LTP rather than just lowering the threshold for obtaining LTP (that is, low levels of NMDA activity during the incubation of slices infected with CaMKII(1–290)VV producing LTP).

If the CaMKII(1–290)-induced synaptic enhancement is LTP, then subsequent protocols that normally induce LTP should yield no further synaptic potentiation. Indeed, a protocol that generated robust potentiation in BGVV-infected slices resulted in no persistent potentiation in slices infected with CaMKII(1–290)VV, suggesting that increased postsynaptic CaMKII activity saturates a process normally used to express LTP.

The absence of LTP in CaMKII(1–290)VV-infected slices could be due to a block rather than occlusion of LTP. We think this is not likely because (i) an increase in CaMKII activity similar to that observed in the CaMKII(1–290)VV-infected slices is observed during LTP (*20*) and (ii) block of CaMKII activity prevents LTP (*10*). Thus, the simplest interpretation of our results is that the absence of LTP in CaMKII(1–290)VV-infected slices indicates a prior maximal activation rather than a block of LTP.

Our results support a model in which postsynaptic CaMKII activity is necessary and sufficient to generate LTP in CA1 hippocampus (*31*). These results, however, are not inconsistent with important roles for other kinases in the generation of LTP (*6–9*). CaMKII could increase quantal size by phosphorylation of postsynaptic GluR1 (*32*) or increase quantal content by activation of GluR1 receptors at synapses with no active GluR1 receptors or by modification of presynaptic function mediated by a retrograde message.

REFERENCES AND NOTES

1. R. A. Nichols, T. S. Sihra, A. J. Czernik, A. C. Nairn, P. Greengard, *Nature* **343**, 647 (1990); C. Baitinger, J. Alderton, M. Poenie, H. Schulman, R. A. Steinhardt, *J. Cell Biol.* **111**, 1763 (1990); J. A. Wagner *et al.*, *Nature* **349**, 793 (1991); M. E. Cohen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8990 (1990); T. Lorca *et al.*, *Nature* **366**, 270 (1993). Reviewed in P. I. Hanson and H. Schulman, *Annu. Rev. Biochem.* **61**, 559 (1992).
2. P. T. Kelly, T. L. McGuinness, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 945 (1984); M. B. Kennedy, M. K. Bennett, N. E. Erondu, *ibid.* **80**, 7357 (1983); J. R. Goldenring *et al.*, *J. Neurochem.* **42**, 1077 (1984).
3. J. E. Lisman and M. A. Goldring, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5320 (1988); S. M. Miller and M. B. Kennedy, *Cell* **44**, 861 (1986).
4. G. L. Collingridge, S. J. Kehl, H. McLennan, *J. Physiol. (London)* **334**, 33 (1983); E. W. Harris, A. H. Ganong, C. W. Cotman, *Brain Res.* **323**, 132 (1984).
5. A. B. MacDermott, M. L. Mayer, G. L. Westbrook, S. J. Smith, J. L. Barker, *Nature* **321**, 519 (1986); W. G. Regehr and D. W. Tank, *ibid.* **345**, 807 (1990); W. Muller and J. A. Connor, *ibid.* **354**, 73 (1991); S. Alford, B. G. Frenguelli, J. G. Schofield, G. L. Collingridge, *J. Physiol.* **469**, 693 (1993); D. J. Perkel, J. J. Petrozino, R. A. Nicoll, J. A. Connor, *Neuron* **11**, 817 (1993); R. Malinow, N. Otmakhov, K. Blum, J. Lisman, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8170 (1994).
6. R. F. Akers and A. Routtenberg, *Brain Res.* **334**, 147 (1985); K. G. Reyman, U. Frey, R. Jork, H. Matthies, *ibid.* **440**, 305 (1988); R. Malinow, D. V. Madison, R. W. Tsien, *Nature* **335**, 820 (1988).
7. R. Malenka, D. V. Madison, R. A. Nicoll, *Nature* **321**, 695 (1986); G. Y. Hu *et al.*, *ibid.* **328**, 426 (1987).
8. T. J. O'Dell, E. R. Kandel, S. G. Grant, *ibid.* **353**, 558 (1991).
9. C. Charriaut-Marlangue, S. Otani, C. Creuzet, Y. Ben-Ari, J. Loeb, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10232 (1991).
10. R. Malinow, H. Schulman, R. W. Tsien, *Science* **245**, 862 (1989); R. C. Malenka *et al.*, *Nature* **340**, 554 (1989).
11. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *Science* **257**, 201 (1992).
12. R. Dingledine, Ed., *Brain Slices* (Plenum, New York, 1984).
13. Transverse hippocampal slices (400 μM thick) from 9- to 13-day-old rats were prepared by the chopper method, subfused with oxygenated artificial cerebral-spinal fluid, and placed in a humidified oxygenated chamber. Multiple injections (~50 per site, 5 to 10 sites) at low pressures (5 to 10 psi for 3 to 6 ms with a General Valve picospritzer through a patch micropipette, ~10-megohms resistance with internal solution) produce the least tissue damage and the greatest delivery of fluid to the extracellular space. To obtain good expression, it is critical that virus-rich solution permeate the tissue, and not exit through injection sites. The health of infected slices (as judged by the ability to obtain whole-cell recordings from neurons with resting potentials) degrades with time equally when injected with solutions containing or not containing virus.
14. D. L. Pettit, S. Perlman, R. Malinow, data not shown.
15. O. Steward, *Cold Spring Harbor Symp. Quant. Biol.* **48**, 745 (1983); _____ and T. M. Reeves, *J. Neurosci.* **8**, 176 (1988); O. Steward and C. E. Ribak, *ibid.* **6**, 3079 (1986); O. Steward and P. Falk, *J. Comp. Neurol.* **314**, 545 (1991).
16. DNA encoding CaMKII(1–290) was inserted into the Sal I–Sma I site in the VV vector pSC-65 downstream from a strong synthetic early-late VV promoter. This vector also contains a gene for β-galactosidase driven by the p7.5 vaccinia promoter. Homologous recombination of the plasmid and wild-type VV was carried out in RK13 cells. Recombinant virus was selected by plaque assays of virus-infected thymidine kinase–negative (TK^-143) cells in the presence of 5-bromo-2′-deoxyuridine (BrdU) (*32*) and X-gal. Large stocks of virus were made by infection of RK13 cells (*32*). All viral stocks were purified by centrifugation over 38% sucrose before use.
17. Y. Yamagata, A. J. Czernik, P. Greengard, *J. Biol. Chem.* **266**, 15391 (1991); C. R. Lin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5962 (1987).
18. M. S. Kapiloff, J. M. Mathis, C. A. Nelson, C. R. Lin, M. G. Rosenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3710 (1991).

19. Slices were made from 9- to 13-day-old rats, and the CA1 region was injected with either BGVV or CaMKII(1–290)VV. The slices were incubated for 6 hours at 33° to 35°C. After incubation, the CA1 region was dissected and homogenized in buffer on ice. For the CaMKII assay, homogenization buffer contained 10 mM tris-HCl (pH 7.4), 1 mM EGTA, 0.5 mM dithiothreitol (DTT), 0.1 mM phenylmethanesulfonyl fluoride (PMSF) (20 μg/ml), leupeptin (5 μg/ml), and soybean trypsin inhibitor (20 μg/ml). Assays for CaMKII activity were carried out by adding an equal volume of assay buffer [100 μM syntide-2, 50 mM Hepes (pH 7.4), 20 mM MgCl, 1 mM DTT, 200 μM adenosine triphosphate (ATP) ([γ-^{32}P]ATP, 50 μCi/ml), 10 μM PKI(6–22)-amide, and 4 μM PKC(19–36)] to the homogenate and incubating at 30°C for 1 min. To determine the percent of Ca^{2+}-dependent phosphorylation, we performed the reaction in the presence of 2 mM Ca^{2+} and 3 μM calmodulin. The Ca^{2+}-independent phosphorylation was determined by adding 5 mM EGTA to the assay buffer. The homogenization buffer for PKC assays contained 20 mM tris-HCl (pH 7.5), 0.5 mM EGTA, 0.5 mM EDTA, 0.5% Triton X-100, aprotinin (25 μg/ml), and leupeptin (25 μg/ml). Assays were carried out by adding an equal volume of assay buffer [25 μM Ac-MBP(4–14), 20 mM tris (pH 7.5), 20 mM MgCl, 10 mM EGTA, 100 μM ATP ([γ-^{32}P]ATP, 50 μCi/ml), ± 20 μM PKC(19–36)] to the homogenate. For the PKA assay tissue was homogenized in buffer containing 50 mM tris-HCl (pH 7.5), 5 mM EDTA, and 0.5% Triton X-100. PKA assay buffer was 50 μM Kemptide, 50 mM tris (pH 7.5), 10 mM MgCl, bovine serum albumin (1 mg/ml), 100 μM ATP ([γ-^{32}P]ATP, 50 μCi/ml), ± 1 μM PKI(6–22)-amide). For PKC and PKA assays, tubes containing the inhibitor peptide were incubated at room temperature for 15 to 20 min to allow time for the peptide to bind. For PKC and PKA assays, reaction tubes were incubated at 30°C for 12 min. For all assays, the reactions were stopped by spotting half of the reaction mixture onto phosphocellulose disks and washing them in 1% phosphoric acid to remove unincorporated [γ-^{32}P]ATP. Initial experiments showed that the amount of radiolabeled phosphate incorporation was linear between 30 s and 16 min, and rate of phosphorylation was linear with protein concentrations of 0.29 to 12 μg/ml. Two to 9 μg were used for all assays. All measurements were made in triplicate. Basal PKC and PKA activities were calculated as the difference between phosphorylation of the substrate peptide in the presence and absence of inhibitor peptide. Average Ca^{2+}-independent activity in the absence of infection was 22 ± 1.8%. Slices, as well as cultured cells infected with BGVV, often showed a slightly increased level of kinase activity compared with uninfected tissue. This increase was not statistically significant and did not affect any monitored electrophysiological measure.
20. K. Fukanaga, L. Stoppini, E. Miyamoto, D. Muller, *J. Biol. Chem.* **268**, 7863 (1993).
21. B. L. McNaughton, C. A. Barnes, P. Andersen, *J. Neurophysiol.* **46**, 952 (1981); M. Raastad, J. F. Storm, P. Andersen, *Eur. J. Neurosci.* **4**, 113 (1992).
22. R. Malinow and R. Tsien, *Nature* **346**, 177 (1990).
23. The lowest stimulus used for each recording was the maximal stimulus that elicited an average transmission of less than 5 pA. This stimulus value varied for different experiments (Fig. 2, B and C) but was not significantly different in the groups infected with CaMKII(1–290)VV or BGVV (BGVV, 16 ± 2 V, n = 14 compared with CaMKII(1–290)VV, 17 ± 2.5 V, n = 16, $P > 0.75$; this includes data with and without APV). From this subthreshold value, stimulus strength was increased in 1- to 2-V increments. We attempted extracellular field recording experiments, but we were unable to get reliable synaptic potentials possibly because of the young age of these animals and the injection and incubation protocols.
24. W. Muller, J. J. Petrozzino, L. C. Griffith, W. Danho, J. A. Connor, *J. Neurophysiol.* **68**, 2264 (1992).
25. T. V. P. Bliss, G. V. Goddard, M. Riives, *J. Physiol. (London)* **334**, 475 (1983); B. L. McNaughton, R. M. Douglas, G. V. Goddard, *Brain Res.* **157**, 277 (1978).
26. R. C. Malenka, D. V. Madison, R. A. Nicoll, *Nature* **319**, 774 (1986); K. G. Reyman, H. K. Matthies, U. Frey, V. S. Vorobyev, H. Matthies, *Brain Res. Bull.* **17**, 291 (1986); F. Asztely, E. Hanse, H. Wigstrom, B. Gustafsson, *Synapse* **11**, 342 (1992); M. Weisskopf, R. Zalutsky, R. Nicoll, *Nature* **362**, 423 (1993); B. L. McNaughton, *J. Physiol. (London)* **324**, 249 (1982).
27. B. Gustafsson, Y. Y. Huang, H. Wigstrom, *Neurosci. Lett.* **85**, 77 (1988).
28. A short baseline period was used to prevent washout of LTP (*22*), which could theoretically occur at shorter times in CaMKII(1–290)VV-infected slices. To ensure stable transmission, we stimulated afferents for ~40 trials after gaining cell-attached patch configuration before gaining whole-cell access. The stability of transmission in the test and control slices after the pairing protocol attests to the generally stable conditions. Furthermore, the blind manner of conducting these experiments prevents bias possibly introduced by deciding when transmission is stable.
29. We cannot rule out the possibility that the CaMKII activity introduced by the virus is having effects on targets not accessible by endogenous CaMKII. However, presence of the much larger protein β-galactosidase in dendrites indicates that CaMKII(1–290) should readily reach synaptic targets.
30. Although comparisons among groups of slices is less reliable than within-slice comparison, the experiments were conducted in a blind fashion. This approach prevents any bias possibly introduced by handling of the slices, the relative positioning of recording and stimulating electrodes, or determination of subthreshold and minimal stimulus levels.
31. Although similar arguments have been put forth to support such a role for PKC, we feel the case is weaker for this enzyme because (i) postsynaptic injection of PKC was not shown to prevent further LTP (*7*) and (ii) experiments in which phorbol esters occluded LTP (*7*) a high drug concentration was used; lower concentrations appear not to occlude LTP [(*27*); D. Muller, J. Turnbull, M. Baudry, G. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6997 (1988)].
32. E. McGlade-McCulloh, H. Yamamoto, S.-E. Tan, D. A. Brickey, T. R. Soderling, *Nature* **362**, 640 (1993).
33. M. Mackett, G. L. Smith, B. Moss, *DNA Cloning: A Practical Approach*, D. M. Glover, Ed. (IRL, Oxford, 1985).
34. We thank J. Lisman, H. Cline, and the members of the Malinow and Perlman laboratories for helpful comments and technical assistance; A. Silva for help with protein kinase assays; E. Kandel for communication of unpublished data; R. Mauer for providing GH3 cells and constructs necessary for luciferase-based assays of CaMKII activity; and B. Moss for vaccinia viruses and vectors.

19 April 1994; accepted 14 September 1994

TECHNICAL COMMENTS

Seasonal Precipitation Timing and Ice Core Records

C. D. Charles *et al.* performed global circulation model experiments of moisture source changes in Greenland (*1*). Their results speak to the risk of interpreting records of isotopic shifts strictly as "temperature," because changes in the location of dominant moisture sources for Greenland precipitation probably changed the effective $\delta^{18}O$-temperature relationship temporally and spatially between the last glacial maximum (LGM) and the present. In light of our preliminary findings from the GISP2 core, we would like to comment on the interpretation of the results of Charles *et al.* and urge caution in how they are applied because they have important implications for paleoclimate reconstruction from ice cores.

Local temperature does play an important role in $\delta^{18}O$ variations in polar ice cores (*2*). Such changes are usually interpreted as indicative of global climate change. The validity of this approach is borne out by the good agreement among paleotemperature records derived from ice cores, ocean sediments, terrestrial pollen records, and so forth. Yet, local isotope values may change as a result of factors other than temperature. Moisture source variability is undoubtedly one of these factors, but of potentially comparable importance is the seasonal variation in the timing of precipitation events, regardless of the source region. Unfortunately, the necessary sensitivity tests have not been run on the global circulation model to examine the importance of this factor (*3*).

Any atmospheric constituent that exhibits large seasonal changes and relatively small long-term changes will be sensitive to the seasonal timing of precipitation (*4*).

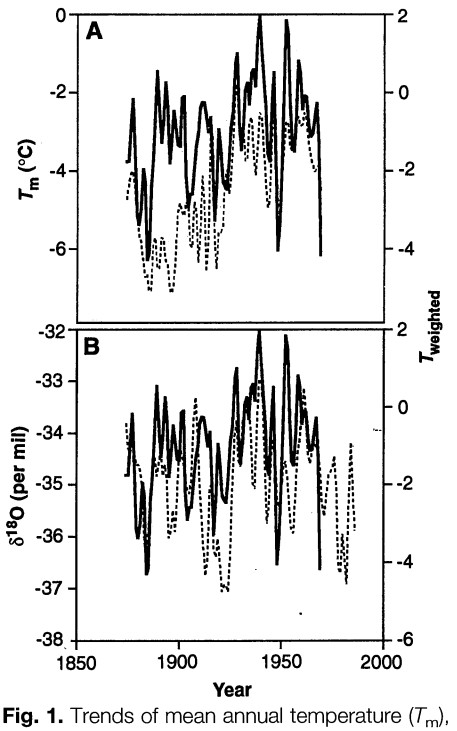


Fig. 1. Trends of mean annual temperature (T_m), accumulation-weighted temperature ($T_{weighted}$), at Jakobshavn, and $\delta^{18}O$ at Summit, Greenland, 1874 to 1970. (**A**) T_m (dashed line) compared with $T_{weighted}$ (bold line). (**B**) $\delta^{18}O$ (dashed line) compared with $T_{weighted}$ (bold line).