The role of calcium–calmodulin kinase II in three forms of synaptic plasticity

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Background: Calcium influx into postsynaptic dendritic spines can, depending on circumstances, activate three forms of synaptic plasticity: long-term potentiation (LTP), short-term potentiation (STP) and long-term depression (LTD). The increased postsynaptic calcium concentrations that trigger all three forms of plasticity should activate the α isoform of calcium–calmodulin kinase type II (αCaMK II), which is present at high levels just below the postsynaptic membrane. Earlier experiments have implicated αCaMK II in the regulation or induction of LTP, but no information is available on the possible role of this enzyme in the two other forms of synaptic plasticity, STP and LTD.

Results: We used mice that lack the gene for αCaMK II to investigate the role of this enzyme in synaptic plasticity. Field potential recordings from hippocampal slices taken from mutant mice show that STP and LTD are, like LTP, absent or markedly attenuated in the absence of αCaMK II. A brief form of synaptic modification — post-tetanic potentiation (PTP) — is, however, intact in the absence of this enzyme.

Conclusions: It appears likely that αCaMK II is involved in the production or global regulation of all three forms of synaptic plasticity. We propose that the activation of this enzyme is a common step in the induction of LTP and STP, and that αCaMK II activity is required for the normal production of LTD.

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Background

Of the three related forms of synaptic plasticity that have been described at hippocampal synapses — long-term potentiation (LTP), short-term potentiation (STP) and long-term depression (LTD) — LTP has received the most attention. This interest in LTP derives, at least in part, from the recognition that it is a potential substrate for certain forms of learning and memory [1]. In addition, LTD has recently been attracting considerable attention [2], because it also has properties that could suit it for a role in memory. STP, which lasts for only about five to thirty minutes (an average of about five minutes under the conditions of our experiments), is the least well understood of the three [3], and could also be involved in the more short-term forms of information storage.

All three types of plasticity share a common trigger step: the influx of calcium through *N*-methyl-D-aspartate (NMDA) receptor channels (although other calcium sources can substitute under some experimental conditions). LTP can be selectively blocked by a variety of kinase inhibitors [4–6] and some other pharmacological agents, and LTD is selectively blocked by certain phosphatase inhibitors [7,8]. STP remains the least well defined of the phenomena, in part because no pharmacological treatments are available that block it selectively, leaving the other forms of plasticity intact. In fact, such a blocking agent might not exist: it is possible that STP is a necessary step in the pathway to LTP, and if this were true, any drug that blocked STP would also block LTP.

Mice lacking the α isoform of calcium–calmodulin kinase type II (αCaMK II) exhibit little or no LTP. They are also severely impaired in their ability to learn certain spatial tasks, despite the fact that no anatomical brain abnormalities are apparent, the Schaffer collateral connections are present, synaptic transmission is intact and NMDA receptor function is normal [9,10]. The influx of calcium through NMDA receptor channels — a trigger for all three forms of plasticity — must, at least under some circumstances, activate the αCaMK II that is present very close to the postsynaptic membrane bearing the NMDA receptors [11–13]. Are the other forms of plasticity affected by the absence of this kinase? The goal of our experiments has been to determine whether STP and LTD are also altered by the absence of αCaMK II.

Our general strategy in these and related experiments is to identify proteins that are thought likely, on the basis of pharmacological and other types of information, to be involved in the network of second messenger pathways that participate in the various forms of synaptic plasticity. We hope to define this network by systematically eliminating such proteins. More specifically, our goals are to define which second messenger systems participate in each form of synaptic plasticity, to determine whether the role is essential or modulatory, and to establish the location of a particular signaling pathway in the overall

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network of second messenger systems that are important for the various synaptic plasticity mechanisms.

Clearly no one experiment will be sufficient to define the role of a particular protein, but the results from each knockout mouse suggest hypotheses about the function of the protein that can be tested in subsequent experiments. Although one must always be careful when interpreting experiments on mutant animals that lack specific enzymes or other proteins involved in signaling systems, this approach can potentially identify the regulatory cascades that are responsible for the synaptic processes underlying learning and memory.

Results

We shall describe our studies of LTD and STP, and some observations on LTP in mice that lack αCaMK II. We shall also present data indicating that post-tetanic potentiation (PTP), a very short-lived form of synaptic plasticity, is intact in the mutant mice.

LTD is deficient in the αCaMK II mutant mice

Figure 1 presents the CA1 response size, averaged across twenty hippocampal slices, as a function of time during the experiment for both wild-type mice (Fig. 1a) and mice lacking functional αCaMK II (Fig. 1b); the ordinate plots the slope of the initial phase of the field potential evoked by the stimulation of Schaffer collaterals. After a period of stimulation to establish the baseline, the Schaffer collateral pathway was stimulated once per second for fifteen minutes to produce LTD; the amount of LTD was estimated by measuring the response size about thirty minutes after the induction period (to ensure that the effect we were studying is persistent, as genuine LTD must be). The wild-type mice showed, on average, a substantial LTD, whereas the mutant mice exhibited significantly less, if any, LTD; this can be seen by comparing the averaged data in Figures 1a and 1b. All of the hippocampal slices from the mutant mice showed an initial depression following the low-frequency stimulation that, on average, was as large as the initial depression exhibited by the wild-type mice, and some of the slices from mutant mice showed a persistent depression of 10–20%.

Is the initial depression a sign of LTD, and is the 10–20% depression that is seen in some mutant slices genuine LTD or simply a non-specific decline in synaptic strength that occurs in some experiments? To decide between these alternatives, we carried out LTD experiments on six hippocampal slices from a mutant mouse, firstly in the presence of D-2-amino-5-phosphonovaleric acid (AP-5, 50 μM; an antagonist of the NMDA receptor), and then we repeated the low-frequency stimulation in the same slices after the AP-5 had been washed out. The average synaptic strength as a function of time is presented for two slices in Figure 1c, and the LTD magnitude for six slices in the presence and then the absence of the AP-5 is shown in the inset bar graph. The transient depression that follows the low-frequency stimulation was not obviously altered by the presence of AP-5 (Fig. 1c), so this transient depression is presumably distinct from LTD, which is blocked by AP-5. The persistent depression observed in some slices appeared to be blocked by AP-5 (see Fig. 1c and the inset), although the cumulative histograms of LTD magnitude with and without AP-5 were not significantly different (Kolmogorov–Smirnoff test, $P > 0.1$; histograms not shown). This observation suggests that hippocampal slices from mutant animals may be capable of exhibiting some, albeit reduced, LTD. We shall return to this issue in the discussion.

LTP after priming by low-frequency stimulation

Mice that lack the γ isoform of protein kinase C (γPKC) are, like the αCaMK II mutants, deficient in LTP, but do exhibit substantially normal LTP after a priming period of low-frequency stimulation [14]. This priming phenomenon was not, however, observed in αCaMK II mutant mice (Fig. 1b). The response size was followed for thirty minutes after the low-frequency stimulation had been applied to produce LTD, and then the Schaffer collateral pathway was presented with a tetanic stimulus that produced LTP in the slices from wild-type mice. No LTP resulted, however, in the hippocampal slices taken from the mutant animals (Fig. 1b).

Cumulative histograms of data from the hippocampal slices from wild-type ($n = 22$) and αCaMK II mutant ($n = 21$) mice are presented in Figure 2, where the cumulative probability is plotted as a function of response size. The slices from wild-type mice clearly have more LTD than the slices from the αCaMK II mutant mice (Fig. 2a), although a persistent depression did occur in some of the mutant mice, as noted above. The hippocampal slices from mutant mice also exhibit little or no LTP following the period of low-frequency stimulation (Fig. 1b), whereas the slices from the wild-type mice reveal clear LTP under the same circumstances (Fig. 1a).

Mice that lack γPKC exhibit apparently normal LTP after LTD is induced, and the size of the LTP is independent of the size of the preceding LTD [14]. That is, the amount of LTP elicited cannot be explained by supposing that the size of LTP reflects how far the synapses are from a saturated level of potentiation. This decoupling of LTD/LTP size is illustrated for wild-type mice (Fig. 3a): the size of LTP is uncorrelated with the magnitude of the preceding LTD. The same is true for the αCaMK II mutant mice (Fig. 3b), although no LTP is seen, even when a persistent depression in synaptic strength of approximately 20% precedes the tetanic stimulation.

These observations are summarized in Figure 3c, where the magnitudes of LTD, LTP following LTD, and LTP alone (without preceding LTD) are plotted for hippocampal slices from wild-type and αCaMK II mutant mice. We conclude that the αCaMK II mutant mice completely lack or exhibit significantly diminished LTP and LTD, and

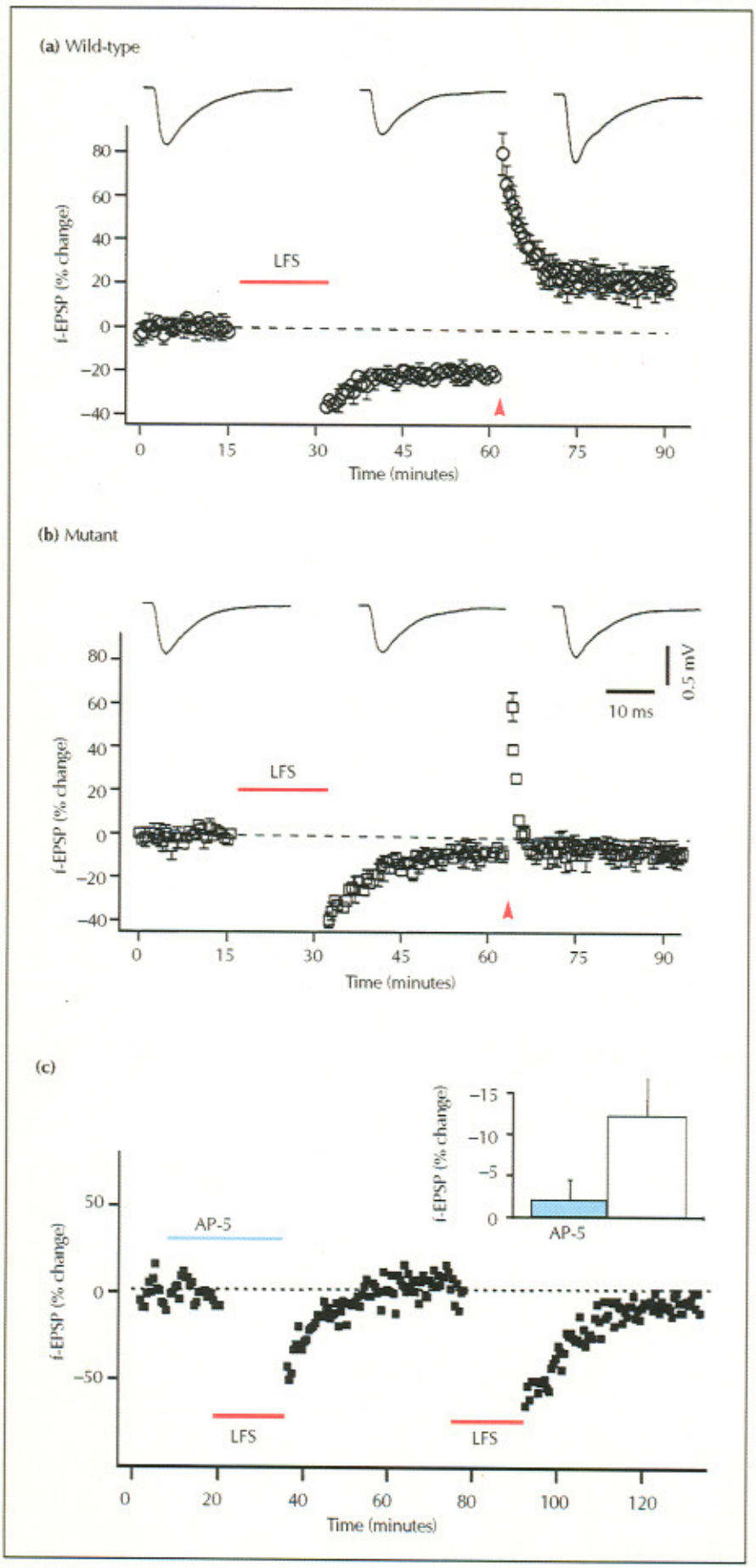

Fig. 1. LTD and LTP in hippocampal slices from **(a)** wild-type and **(b)** mutant mice. The initial slope of the field excitatory postsynaptic potential (f-EPSP), expressed as a percent deviation from the average baseline level, is plotted as a function of time during the experiment. Both data sets are means (± S.E.) of 20 slices. A period of stimulation to establish the baseline response size precedes the red bar marked LFS. LFS indicates an episode of low-frequency stimulation (1 Hz) to induce LTD, and the red arrow indicates the time of a tetanic stimulation to induce LTP. The insets show sample traces taken from the control period, after stable LTD had been achieved, and after LTP had reached a steady level. The calibration marks in (b) apply to both wild-type and mutant slices. **(c)** Use of the specific inhibitor AP-5 to investigate whether the persistent depression exhibited by the slices from mutant mice is NMDA-receptor dependent, and thus a sign of true LTD. Synaptic strength is measured as a percent change in the field EPSP slope, as a function of time during the experiment. AP-5 (50 μM) was present as indicated by the blue bar, and two periods of low frequency stimulation, one in the presence of AP-5 and one after the AP-5 was washed out, are indicated by the red horizontal lines just above the time axis. This figure shows the mean of two slices for which the experimental procedures were identical; data from four other slices were not included in the mean because AP-5 application was for a different duration or the washout period was different. The inset bar graph presents the mean (± S.E.) for LTD magnitude in the presence of AP-5 (blue bar) and after washout of AP-5 (open bar) from six slices.

that they do not exhibit LTP following low-frequency stimulation as do wild-type and γPKC mutant animals.

STP is absent in the αCaMKII mutant mice

Following a tetanic stimulus, the response size recorded from populations of CA1 neurons is increased, and then declines in at least two phases to reach the final steady level of synaptic strength. The initial phase of decline is known as PTP, and the subsequent and slower relaxation is identified as STP [15]. STP depends upon postsynaptic calcium influx [16,17], whereas PTP is a purely presynaptic phenomenon [18,19].

Because STP was first isolated in experiments that used pharmacological inhibition of kinase activity — where

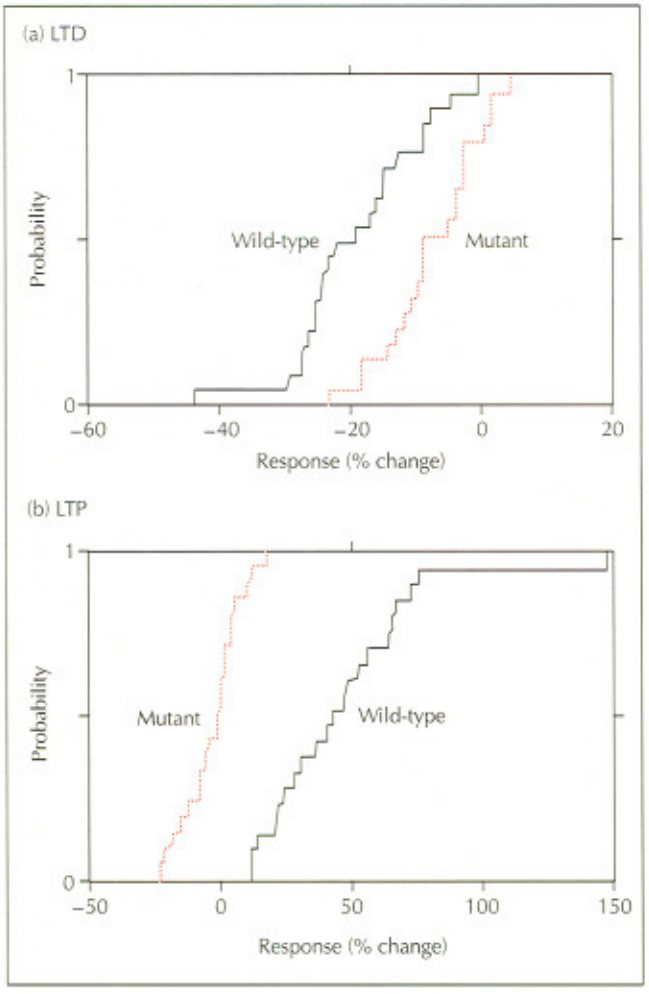

Fig. 2. Cumulative histograms expressing the probability that a hippocampal slice exhibited a synaptic strength less than or equal to the value given on the abscissa (expressed as percent change from the baseline value of field EPSP slope). Data are from slices from wild-type (n = 22) and αCaMKII mutant (n = 21) mice as indicated. **(a)** Synaptic strength following low-frequency stimulation to produce LTD. The two cumulative histograms are significantly different ($p < 0.005$; two-tailed Kolmogorov–Smirnoff test). **(b)** Synaptic strength following a tetanic stimulus given 30 min after the low-frequency stimulation that produced the data shown in (a); $p < 0.001$. Synaptic strength was measured by averaging over a 10 minute period starting 20 minutes after either the low-frequency stimulation or the tetanic stimulation.

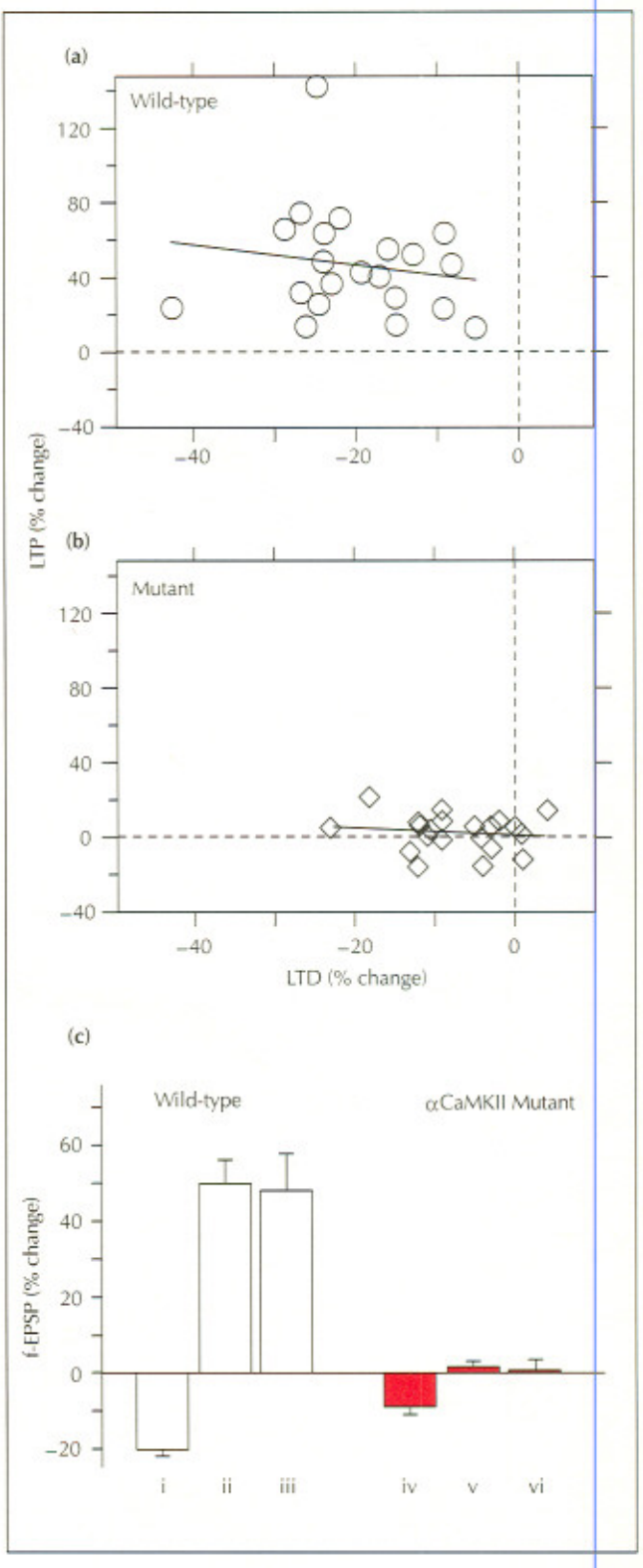

Fig. 3. Size of LTP (expressed as percent change from the baseline response size) as a function of the size of preceding LTD (percent change from the baseline response size) for hippocampal slices from **(a)** wild-type and **(b)** mutant mice (line indicates best fit to data). **(c)** Histogram showing (i) the average amount of LTD (n = 22), (ii) LTP following the induction of LTD (n = 21), and (iii) LTP in slices that had not previously experienced low-frequency stimulation (n = 26), for slices from wild-type mice. (iv–vi) Comparable values for slices from mutant mice: (iv,v) n = 21; (vi) n = 28. (i) is significantly different from (iv), $p < 0.0001$ (Student's t-test, two-sided, unpaired).

STP was seen without a subsequent LTP [4–6] — we felt that it was important to examine STP in the αCaMK II mutant animals. Figure 4 presents data on STP from hippocampal slices of wild-type and mutant mice. Response size is plotted as a function of time after the tetanic stimulus for typical individual experiments (Fig. 4a) and averages across twenty slices (Fig. 4b). The average decay time constant for the STP in wild-type slices is 202.5 seconds. The mutant animals, however, exhibit only what appears to be PTP, with an average time constant of 37.0 seconds, under the stimulation conditions of these experiments (see below). Cumulative histograms show that the mutant animals' slices never exhibit a prolonged potentiation following a tetanic stimulus, whereas wild-type slices always do (Fig. 4c). Thus, the removal of αCaMK II activity by a gene-targeting technique eliminates both LTP and STP, whereas pharmacological interventions intended to inhibit CaMK II block LTP, but leave STP at least partially intact [4,5].

PTP is intact in the αCaMK II mutant mice

The rapid decay in synaptic strength following tetanic stimulation appears to be PTP. Might it, however, actually represent STP with an abnormally rapid time course? To address this question, we have examined the rapidly decaying phases of synaptic plasticity in mutant mice before and after the application of AP-5 to block NMDA receptors. AP-5 should block or attenuate STP [20], so that if the rapidly decaying component of increased synaptic strength is unaffected by the blockade of NMDA receptors, this would be a strong argument for claiming that PTP is responsible for the brief transient increase in synaptic strength seen in the mutants.

We applied a tetanic stimulation to hippocampal slices from mutant animals before (filled circles) and after (open circles) the application of 15 μM AP-5 and measured the initial field potential strength (Fig. 5); these data are from averages across the 13 slices from which data were obtained before and after the application of AP-5. The peak post-tetanic synaptic strength was nearly the same before and after the application of AP-5 (63% versus 65% increase), and the time constant for decay was also very similar (24 versus 20 seconds). Average data from seven wild-type slices (after the application of AP-5) are also presented (open triangles in Fig. 5). The time constant for the wild-type slices was 22 seconds, which is quite close to the value for the mutant slices. Note that the time constant for PTP is about half that given in the preceding section for the mutant slices (Fig. 4b), and the initial amplitude is different. We are uncertain why the values are different in the two sets of slices from mutant animals, although both values are within the usual range for PTP decay times. Two possibilities are, first, that the differences result from the use of seven-month old mice in the PTP experiments, but one to three month-old mice in the other experiments described above; and second, that they are due to the use of a slightly different stimulation protocol for the PTP experiments (see

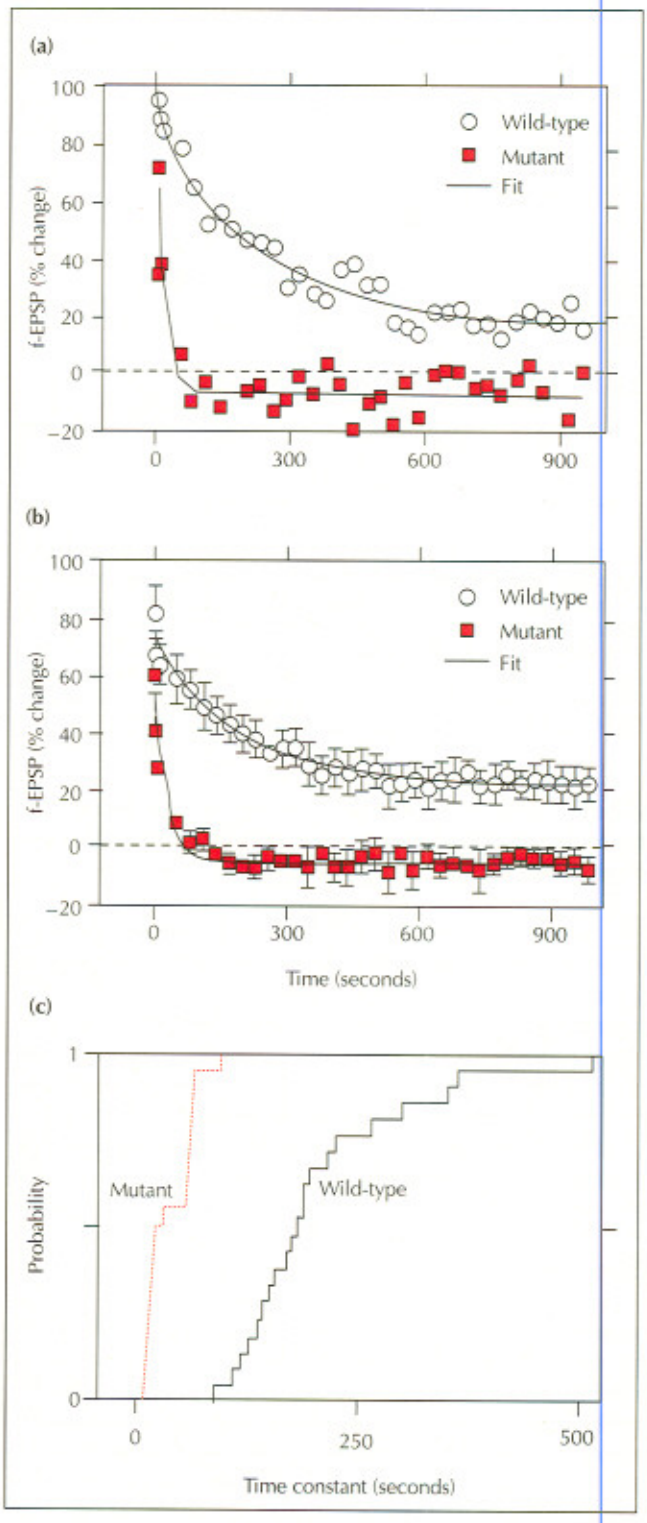

Fig. 4. Initial slope of field excitatory postsynaptic potential (EPSP) as a function of time after a tetanus for hippocampal slices from wild-type (open circles) and αCaMK II mutant (red squares) mice. **(a)** Individual slices: time constants for the smooth curves fitted to the data points are 213.8 seconds for the wild-type slice and 34.5 seconds for the mutant slice. **(b)** Averages across 20 slices: the curve fitted to the wild-type data has a time constant of 202.5 seconds, and for the mutant data, 37 seconds. **(c)** Cumulative probability histograms for the distribution of measured time constants for individual experiments (mutant and wild-type slices, as labeled). The two cumulative histograms are significantly different, $p < 0.001$ (Kolmogorov–Smirnoff test).

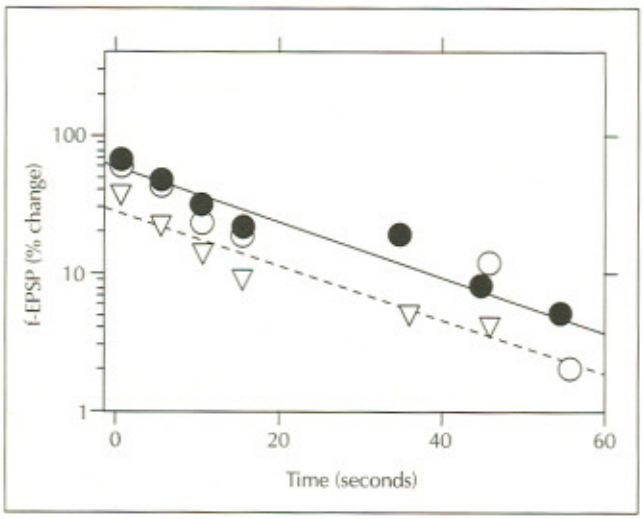

Fig. 5. Semi-logarithmic plot of post-tetanic synaptic strength (measured as initial slope of field excitatory postsynaptic potential, percent change from the final steady value) as a function of time after the tetanus. Filled circles: αCaMK II mutant slices without AP-5; time constant 23.8 seconds (n=13). Open circles: mutant slices with 15 μM AP-5; time constant 20.4 seconds (n=13; same slices as for filled circles). Open triangles: wild-type slices with 15 μM AP-5; time constant 22.2 seconds (n=7).

Materials and methods; this was because we found that the older mice exhibited a transient depression following the stronger tetanic stimulation).

The initial value for the PTP is less for the slices from wild-type mice than that for slices from the mutant animals (see Fig. 5), but comparable variations in the magnitude of PTP are frequently observed from one group of slices to the next. We conclude, therefore, that the brief transient increase in synaptic strength is indeed PTP, and that PTP is thus not diminished in hippocampal slices from mutant animals, unlike the other forms of plasticity studied in these experiments.

Discussion

Although the very short form of synaptic plasticity, PTP, appears to be intact in the αCaMK II mutant mice, STP and LTP seem to be absent and LTD is, at least, significantly reduced. This is, we believe, the first time that the action of a protein kinase has been implicated in LTD and STP, as pharmacological experiments have reported that STP is still present after kinase inhibition [4–6], and only the action of a phosphatase has been implicated in LTD [7,8].

Unfortunately, we cannot firmly conclude that some LTD remains in slices from the mutant animals. In the LTD experiments using AP-5, we had only a single animal available and so we used each slice as its own control. That is, we first applied AP-5, carried out the low-frequency stimulation, waited thirty minutes for the response to stabilize, washed out the AP-5, and then repeated the low-frequency stimulation. If we could be sure that the presence or absence of AP-5 was the only relevant variable operating in these experiments, it would appear that genuine LTD is sometimes seen in the mutant animals, because the persistent depression following low-frequency stimulation does not appear when AP-5 is present. The use of each slice as its own control means that sequence and temporal factors, rather than the presence or absence of AP-5, could have been the relevant variables in determining the amount of persistent depression. We are confident that LTD is diminished in slices from the mutant animals, but we cannot be sure that some LTD is present; LTD might therefore also be absent in the mutant animals.

It is interesting to compare this αCaMKII mutant mouse with the γPKC-deficient mouse, which has LTD intact but exhibits LTP only if the tetanic stimulus is preceded by a period of low-frequency stimulation of the sort used to produce LTD [14]. Clearly, γPKC is not required for plasticity, and so it must be regulatory. The pattern resulting from its deficiency is what would be expected for an enzyme placed in a regulatory network at a point after the control of LTD and LTP have diverged, whereas αCaMKII seems — but is certainly not proven — to have a position early in the regulatory network, or plasticity cascade, before control of the two forms of plasticity has become separate. Thus, certain enzymes (for example, αCaMKII) have global influence over all three types of synaptic plasticity, whereas others (for example, γPKC) are devoted to the control of only certain forms of plasticity.

Lisman [21] has proposed a detailed theoretical model for LTP and LTD in which multiple kinase and phosphatase cascades converge on postsynaptic αCaMKII to regulate synaptic strength, and Mulkey and colleagues [7,8] have recently provided evidence that supports components of this model. One clear prediction of the Lisman scheme is that, because Hebbian and anti-Hebbian regulation of synaptic strength involve the phosphorylation state of αCaMKII, production of both LTD and LTP should depend on the presence of this enzyme and thus should be absent if the enzyme is absent or pharmacologically inactivated. The data we presented here can also be considered as support for the Lisman theory, at least the part of it that identifies αCaMKII as a key to synaptic plasticity.

Materials and methods

The methods for preparation of slices and electrophysiological recordings have been described before [22]. Briefly, transverse hippocampal slices (350–400 μm) were obtained from αCaMKII homozygous mice [9] or their normal littermates and maintained in an incubation chamber. In the present study, mice were between one to three months old, except in the PTP study, in which they were around seven months old; this was merely because of the availability of mice at the time experiments were performed. The wild-type mice in the PTP study were the C57/Bl-6J strain, because normal littermates were not available at the time experiments were performed.

A single hippocampal slice rested in a submerged recording chamber perfused continuously with artificial cerebrospinal fluid (ACSF) at a rate of ~2 ml min^{-1} at a temperature of 31.5 ± 0.5 °C. The ACSF was equilibrated with 95% O_2/5% CO_2, and was composed of: NaCl, 120 mM; KCl, 3.5 mM; NaH_2PO_4, 1.25 mM; $NaHCO_3$, 26 mM; $MgCl_2$, 1.3 mM; $CaCl_2$ 2.5 mM. Extracellular field excitatory postsynaptic potentials (f-EPSPs) were recorded in the apical dendrite layer of CA1 with electrodes (1–2 MΩ) filled with ACSF. Bipolar tungsten stimulating electrodes were used to apply voltages (100 μs duration) to Schaffer collateral–commissural afferents at 30 s intervals in order to evoke f-EPSPs. A shorter stimulus interval (5–10 seconds) was used for the first 1–2 minutes after tetanus. The stimulus intensity was adjusted to evoke baseline responses of similar sizes for all the slices. Recordings were performed with an Axopatch-1A (Axon Instruments).

The values for LTP or LTD were typically taken between 20–30 minutes after tetanus or low-frequency stimulation. In some cases, responses after tetanus or low-frequency stimulation were observed for longer than 30 minutes to ensure no further changes in response sizes occurred. STP typically lasted less than 15 minutes after tetanus under our conditions. The tetanus was composed of five bursts — each at 100 Hz and 200 ms, with an interburst interval of 10 seconds. The protocol for inducing LTD was to stimulate slices at 1 Hz for 15–18 minutes.

To distinguish PTP from STP, the responses taken shortly after tetanus were compared in the presence or absence of AP-5 in the same slices. AP-5 was given either before and during the first tetanus, or before and during the second tetanus; no discernible difference was observed in the amount of PTP or STP produced by tetanus regardless of the order of perfusion of AP-5. The tetanus for PTP study was reduced to two to three bursts; STP and LTP could be reliably induced in this age group of wild-type mice.

Electrical signals were filtered at 1 kHz, digitized at 5 kHz, collected and analyzed with programs written in QuickBASIC/AxoBASIC. AP-5 was from Cambridge Research Biochemicals.

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References

1. Bliss TVP, Collingridge GL: **A synaptic model of memory: long-term potentiation in the hippocampus.** *Nature* 1993, **361**:31–39.
2. Dudek SM, Bear MF: **Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade.** *Proc Natl Acad Sci USA* 1992, **89**:4363–4367.
3. Hannay T, Larkman A, Stratford K, Jack J: **A common rule governs the synaptic locus of both short-term and long-term potentiation.** *Curr Biol* 1993, **3**:832–841.
4. Malinow R, Schulman H, Tsien RW: **Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP.** *Science* 1988, **245**:862–866.
5. Reymann KG, Brödemann R, Kase H, Matthies H: **Inhibitors of calmodulin and protein kinase C block different phases of hippocampal long-term potentiation.** *Brain Res* 1988, **461**:388–392.
6. O'Dell TJ, Kandel ER, Grant SGN: **Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors.** *Nature* 1991, **353**:558–560.
7. Mulkey RM, Herron CE, Malenka RC. **An essential role for protein phosphatases in hippocampal long-term depression.** *Science* 1993, **261**:1051–1055.
8. Mulkey RM, Endo E, Shenolkar S., Malenka RC: **Involvement of calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression.** *Nature* 1994, **369**:486–488.
9. Silva AJ, Stevens CF, Tonegawa S, Wang Y: **Deficient hippocampal long-term potentiation in α-calcium–calmodulin kinase II mutant mice.** *Science* 1992, **257**:201–206.
10. Silva AJ, Paylor R, Wehner JM, Tonegawa S: **Impaired spatial learning in α-calcium-calmodulin kinase II-mutant mice.** *Science* 1992, **257**:206–211.
11. Carlin RK, Grab DJ, Cohen RS, Siekevitz P: **Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities.** *J Cell Biol* 1980, **86**:831–843.
12. Kennedy MB, Bennett MK, Erondu NE: **Biochemical and immunochemical evidence that the 'major postsynaptic density protein' is a subunit of a calmodulin-dependent protein kinase.** *Proc Natl Acad Sci USA* 1983, **80**:7357–7361.
13. Ocorr KA, Schulman H: **Activation of multifunctional Ca^{2+}/calmodulin-dependent kinase in intact hippocampal slices.** *Neuron* 1991, **6**:907–914.
14. Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S: **Modified hippocampal long-term potentiation in PKCγ-mutant mice.** *Cell* 1993, **75**:1253–1262.
15. Malenka RC: **Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus.** *Neuron* 1991, **6**:53–60.
16. Kullmann DM, Perkel DJ, Manabe T, Nicoll RA: **Ca^{2+} entry via postsynaptic voltage-sensitive Ca^{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus.** *Neuron* 1992, **9**:1175–1183.
17. Malenka RC, Lancaster B, Zucker RS: **Temporal limits on the rise in postsynaptic calcium required for the induction of long-term potentiation.** *Neuron* 1992, **9**:121–128.
18. Magleby KL: **Short-term changes in synaptic efficacy.** In *Synaptic Function.* Edited by Edelman GM, Gall VE, Cowan KM. New York: John Wiley & Sons, Inc.; 1987:21–56.
19. Zucker RS: **Short-term synaptic plasticity.** *Annu Rev Neurosci* 1989, **12**:13–31.
20. Anwyl R, Mulkeen D, Rowan MJ: **The role of N-methyl-D-aspartate receptors in the generation of short-term potentiation in the rat hippocampus.** *Brain Res* 1989, **503**:148–151.
21. Lisman J: **A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory.** *Proc Natl Acad Sci USA* 1989, **86**:9574–9578.
22. Stevens CF, Wang Y: **Reversal of long-term potentiation by inhibitors of haem oxygenase.** *Nature* 1993, **364**:147–149.

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