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Transient protein kinase C activation primes long-term depression and suppresses long-term potentiation of synaptic transmission in hippocampus

(CA1/learning and memory/synaptic plasticity)

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ABSTRACT **Activity-dependent long-lasting plasticity in hippocampus and neocortex includes long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength. Recent studies have confirmed theoretical predictions that the sensitivity of LTP- and LTD-inducing mechanisms is dynamically regulated by previous synaptic history. In particular, prior induction of either repeated short-term potentiations or LTP lowers the threshold for induction of LTD and raises the threshold for LTP. In the current study, transient activation of protein kinase C with phorbol 12,13-diacetate was able to substitute for synaptic activity in priming synapses to exhibit enhanced homosynaptic LTD and to suppress the induction of LTP at Schaffer collateral synapses in area CA1 of hippocampal slices. This priming lasted 30 min, but not 3 hr, following phorbol 12,13-diacetate bath application. These data suggest that a protein kinase C-sensitive phosphorylation site may be an activity-sensitive target mediating the rapid expression of LTP and LTD.**

Activity-driven long-lasting changes in synaptic strength are thought to play an important role in the development of cortical synaptic architecture (1) and adult memory storage (2). To date, the most studied type of neuronal plasticity is long-term potentiation (LTP), a persistent increase in synaptic strength (3) induced by simultaneous, strong presynaptic and postsynaptic excitation (4). However, it has also been suggested that some mechanism for long-term depression (LTD) of synaptic strength may be needed (5, 6) to limit the saturation of LTP, sharpen the storage of time-dependent correlated activity, and, perhaps, regulate the transition of LTP to a more permanent form of synaptic modification. In support of this idea, prolonged presynaptic activity in the absence of postsynaptic depolarization has been found to elicit homosynaptic LTD of hippocampal synaptic strength (7–10).

Many theoretical models of neural network learning employ a bidirectional learning rule, where a sum of pre- and postsynaptic activity over a given threshold evokes LTP, and activity under that threshold induces LTD. A serious defect with such a learning rule is that, with the threshold set to a fixed value, network firing rates can positively feed back to induce either runaway LTP (potentiated synapses are more likely to elicit LTP) or LTD (depressed synapses are more likely to show further LTD), resulting in failure of the network to "learn" input patterns. Typically, this problem has been resolved by implementing an *activity-dependent* sliding threshold of correlated activity levels, where previous high levels of synaptic activity increase the likelihood of LTD and decrease that of LTP (6). The result is to force the average level of induction of synaptic plasticity toward the center of the available range.

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There are recent experimental data that support the idea of a sliding threshold for the induction of long-term changes in synaptic strength. Initial studies found that hippocampal synapses where LTP had been induced could be more reliably depressed (or depotentiated) by low-frequency stimuli (LFS; refs. 11 and 12). Recently, it has been reported that the magnitude of LFS-induced LTD is significantly *greater* following the induction of either short-term potentiation (STP) or LTP (13), and Huang *et al.* (14) reported that induction of STP also homosynaptically *suppresses* the induction of LTP in the hippocampus. Taken together, these studies suggest that the ability to shift the level of stimulation necessary to induce LTD, versus that needed for LTP, is a property of at least some cortical synapses, but say nothing about the neurochemical mechanisms for dynamically changing this threshold level.

A great deal of work has also gone into elucidating the neuronal messenger systems that induce LTP, and, recently, similar studies of LTD have begun. Substantial increases in postsynaptic intracellular [Ca^{2+}] are a necessary trigger for LTP (15), and smaller elevations of intracellular [Ca^{2+}] appear to be required to evoke LTD (10, 16). A number of Ca^{2+}-activated enzymes have been implicated in the induction of LTP, largely through experiments where only partially selective inhibitors of these enzymes reduce the amplitude of LTP. One of many classes of Ca^{2+}-dependent enzymes that is thought to contribute to the full expression of LTP is protein kinase C (PKC; refs. 17–19).

Since activation of PKC seems to be an event that accompanies, and may contribute to, the induction of LTP, I hypothesized that the activation of PKC might also alter the sensitivity of synapses for the subsequent induction of LTD and LTP. In particular, increases in synaptic activity sufficient to activate PKC, but not to induce long-term changes in synaptic strength, could be a signal that enhances the proclivity of a synapse to exhibit LTD, while suppressing the induction of LTP. I tested this hypothesis at Schaffer collateral (SCH)–CA1 synapses in rat hippocampal slices.

MATERIALS AND METHODS

Combined slices of hippocampus and entorhinal cortex (400 μm thick) were prepared from male Sprague–Dawley rats (125–150 g) as described (20), with one exception. Slices were cut simultaneously using a spring-loaded mechanism that rapidly forced a parallel grid of 20-μm diameter wires through the tissue. Immediately after preparation, slices were transferred to a dual-compartment Haas-style interface recording cham-

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; HFS, high-frequency stimulation; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PDiAc, phorbol 12,13-diacetate; PKC, protein kinase C; SCH, Schaffer collateral; STP, short-term potentiation; SUB, subiculum; EPSP, excitatory postsynaptic potential.

ber (21) and incubated at 33–35°C throughout the experiment. The external bathing solution contained 126 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose. Slices were oxygenated by passing humidified 95% O_2/5% CO_2 over the upper surface of the slices plus gassing the external solution prior to perfusion (perfusion rate, 3 ml/min). Extracellular recording electrodes were filled with 2 M NaCl and had a resistance of 1–5 MΩ. Bipolar Teflon-insulated stainless steel wire electrodes were used for stimulation (50-μm tip diameter). Extracellular field potentials (excitatory postsynaptic potentials, EPSPs) were recorded in the CA1 pyramidal cell dendritic layer (stratum radiatum) and evoked by bipolar stimulation of SCH/commissural axons in stratum radiatum. To isolate separate synaptic inputs onto the same population of CA1 pyramidal neurons, one stimulating electrode (SCH) was placed in stratum radiatum on the CA3 side of the recording site, while a second (SUB) was placed in the same relative location on the subicular side of the recording electrode. These inputs were deemed to be independent only if paired-pulse stimulation of neither SCH–SUB nor SUB–SCH at intervals of 20–50 msec elicited any facilitation of the second response. Thereafter, these inputs were alternately stimulated once each 30 sec throughout the remainder of the experiment. Phorbol 12,13-diacetate (PDiAc) was dissolved in distilled H_2O to a stock concentration of 10 mM and then diluted to a final concentration of 1 μM in bathing solution immediately prior to bath application.

RESULTS

If the activation of PKC is an event that accompanies and contributes to the induction of LTP, I hypothesized that the activation of PKC might also be either *necessary* or *sufficient* to lower the stimulus threshold for inducing LTD. To evaluate the latter possibility, I tested whether activation of PKC with PDiAc was sufficient to prime synapses to express enhanced homosynaptic LTD. Fig. 1*A* illustrates one such experiment, where EPSPs recorded extracellularly were alternately evoked each 30 sec by stimulating one of two independent SCH synaptic inputs in *stratum radiatum* of area CA1. First, 1-Hz stimulation of one input was applied for 15 min through a stimulating electrode on the subicular side of the recording electrode (SUB, ▽) and evoked only a small homosynaptic depression (−6%). Then, PDiAc (1 μM) was bath applied for 30 min (hatched box) and elicited a reversible (>85% reversal in all slices) potentiation of synaptic transmission, similar to previous reports (20). After a 30-min drug washout, a 1 Hz × 15 min stimulation of the second input through an electrode on the CA3 side of the recording site (SCH, ●) now elicited marked homosynaptic LTD (−62%). Finally, a second 1 Hz × 15 min stimulation of the previously stimulated SUB input now also evoked significantly larger LTD (−42%) after the transient activation of PKC.

Fig. 1*B* summarizes the studies comparing LTD elicited by low-frequency SCH stimulation (1 Hz × 15 min) in control, untreated slices (open bar, mean ± SEM) versus slices pretreated with 1 μM PDiAc (hatched bar) 45–60 min prior to induction of LTD. Transient activation of PKC more than tripled the magnitude of LTD evoked by low-frequency SCH stimulation (*, $P < 0.05$, Student's t test). One additional, consistent, and interesting observation (Fig. 1*A Inset*) was that when 1-Hz stimulation was given to one of the two synaptic inputs, the subsequent bath application of PDiAc caused a significantly larger, reversible potentiation of synaptic potentials at the unstimulated input (Fig. 1*A Inset*; open bar, mean ± SEM) compared with the one that had been previously stimulated (Fig. 1*A Inset*; hatched bar; *, $P < 0.05$, paired t test compared to unstimulated inputs).

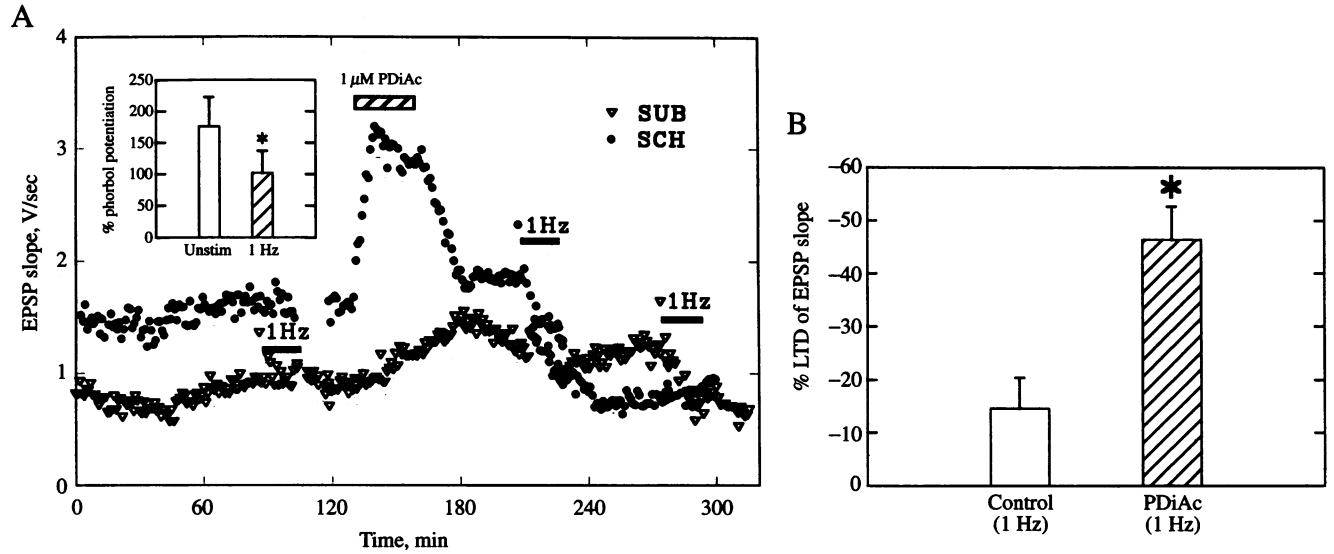

FIG. 1. Transient phorbol ester activation of PKC primes synapses for enhanced LTD of synaptic strength. (*A*) Plot of EPSP slopes (V/sec) versus time evoked at two independent sets of synapses on apical dendrites of CA1 pyramidal neurons in a hippocampal slice. Bipolar stimulating electrodes were placed on the CA3 side (SCH, ●) and the subicular side (SUB, ▽) in stratum radiatum, straddling an extracellular recording pipette in the apical dendritic field of CA1 pyramidal neurons, and population EPSPs were evoked by alternating each 30-sec stimulation of one of the two inputs. Prior to phorbol ester application, a low-frequency stimulus train (LFS, 1 Hz × 15 min) was applied to the SUB input and evoked only a small, homosynaptic depression of synaptic strength. PDiAc (1 μM) was then bath applied and produced a potentiation of synaptic transmission that was smaller at the previously stimulated synapses (*Inset*, *, $P < 0.05$ compared with the unstimulated input) and that largely reversed during the 45-min drug-free wash period. Following drug washout, a second LFS (1 Hz × 15 min) was applied to the SCH input and now elicited a marked homosynaptic LTD (−58%) of synaptic strength. Furthermore, another LFS identical in stimulus intensity to the first LFS was given to the SUB input and now elicited a 50% reduction of synaptic strength. (*B*) Bar graphs of all experiments ($n = 7$) comparing control LTD elicited by LFS (open bar, mean ± SEM) with LTD evoked 45–60 min following activation of PKC with PDiAc (hatched bar, mean ± SEM). LFS evoked significantly larger LTD of synaptic strength in slices where PKC had been activated previously (*, $P < 0.05$, Student's t test) compared with untreated control slices.

My second working hypothesis was that transient activation of PKC could be *sufficient* to *raise* the threshold stimulus needed to induce LTP of synaptic transmission. To test this, I employed the same experimental arrangement as described previously, now bath applying PDiAc for 30 min prior to giving high-frequency θ burst stimulation (HFS; four trains of 100 Hz × five pulse bursts at an interburst frequency of 5 Hz) to induce LTP. Fig. 2*A* illustrates one such experiment, where 1 μM PDiAc was bath applied (hatched box) for 30 min, inducing a reversible potentiation of SCH evoked EPSPs that reversed after 60 min of drug-free wash. HFS (TET) given after 60, 110, and 140 min post-PDiAc application evoked little or no LTP. In contrast, after a total washout period of some 6 hr, a fourth identical HFS did elicit a stable LTP of the EPSP slope, some 160% of pretetanus baseline response.

In some experiments, HFS was applied before and after activating PKC. Fig. 2*B* shows one such experiment, where HFS (TET) given to one SCH input (SUB, ●), evoked homosynaptic LTP measured as 180% of pretetanus baseline responses. Thereafter, PDiAc (1 μM) was bath applied, and potentiated and control pathways showed reversible increases in synaptic EPSPs. Finally, two identical tetani were given to the previous control pathway (SCH, ▽), and no significant LTP was observed. Fig. 2*C* summarizes these experiments, comparing the mean ± SEM percent increase in SCH evoked EPSP slope recorded 30 min posttetanus in field CA1 of control slices (open bar) with slices where PDiAc (1 μM) was bath applied 60–120 min prior to HFS (hatched bar). Transient activation of PKC with PDiAc produced a virtually complete suppression of the induction of LTP by high-frequency SCH stimulation (*, $P < 0.05$, Student's t test compared with control LTP) compared with either control, untreated slices, or separate inputs in the same slice tetanized prior to PDiAc application.

These experiments indicate that, even though phorbol ester induces a largely reversible potentiation of synaptic transmission, phorbol-induced shifts in the thresholds for LTD and LTP outlast the period of PKC potentiation and, presumably, PKC activation. To test whether PKC-sensitive potentiation can, itself, be affected by low-frequency synaptic activity, I examined the effect of LFS applied *during* phorbol ester activation of PKC. As illustrated in Fig. 3, LFS (1 Hz × 15 min) was able to cause only a short-term depression of synaptic strength when applied during bath application of phorbol ester ($n = 3$ slices). Interestingly, this stimulation also converted the actions of phorbol ester into a longer-lasting potentiation of synaptic transmission that persisted well after drug washout. As observed previously, another LFS applied after washout of phorbol ester was again able to elicit stable LTD of synaptic strength.

DISCUSSION

Taken together, these studies support the conclusion that transient activation of PKC phosphorylates a site or sites that can prime synapses for larger induction of homosynaptic LTD and also reduce the ability of these synapses to express LTP. Stimulation of PKC with phorbol ester has previously been shown to markedly potentiate SCH synaptic transmission, but this action reversed upon washout (22). The observed modulation of LTP and LTD appears to outlast the duration of phorbol ester-induced potentiation of synaptic transmission.

Since it has been reported that phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors can increase single channel conductance (23, 24), one reasonable hypothesis is that phosphorylation of this site by PKC may also lead to longer-term shifts in the threshold for synaptic plasticity—that is, while PKC phosphorylates a site that increases AMPA channel conductance,

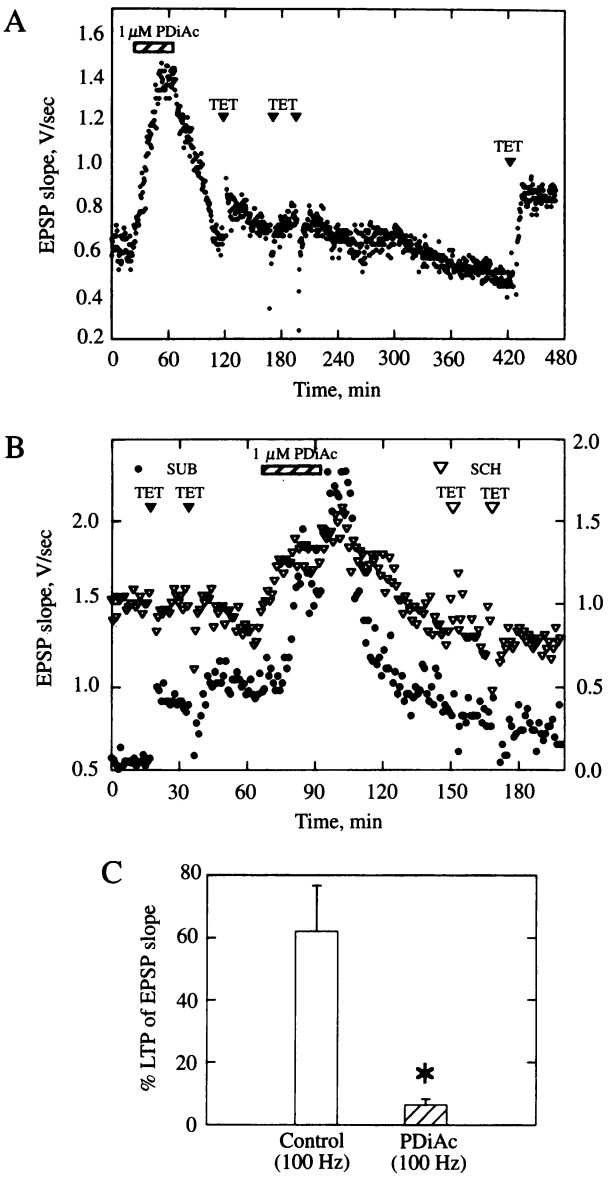

FIG. 2. Transient phorbol ester activation of PKC also suppresses the induction of LTP of synaptic strength. (*A*) Plot of EPSP slopes (V/sec) versus time evoked at one SCH/commissural synaptic input in stratum radiatum of field CA1. PDiAc (1 μM) was bath applied and elicited the typical reversible potentiation of synaptic transmission. After 60 min of drug-free washout, two high-frequency trains (HFS) of θ-burst stimulation (TET, eight 100 Hz × five pulse bursts separated by an interburst interval of 200 msec) were applied and elicited only very small LTP (+7%). Similarly, a second and third HFS given 110 and 140 min into the drug-free wash elicited minimal LTP. In contrast, after a total washout period of 360 min, a fourth HFS did elicit robust LTP, 160% of pretetanus baseline EPSP slope. (*B*) Plot of EPSP slopes (V/sec) versus time evoked at two independent synaptic inputs in field CA1, using the same protocol as in Fig. 1. Prior to application of phorbol ester, two sequential HFS θ-burst stimulus trains were applied to the SUB input (●; left y axis) and elicited robust homosynaptic LTP (+36% increase, then an additional +25%). PDiAc (1 μM) was then bath applied for 30 min, followed by a 60-min drug-free washout. When two identical HFS trains were then applied to the SCH input (▽; right y axis), no LTP was observed. (*C*) Bar graphs of all experiments ($n = 6$) comparing control LTP elicited by HFS (open bar, mean ± SEM) with LTP evoked following activation of PKC with PDiAc (hatched bar, mean ± SEM). HFS evoked significantly less LTP of synaptic strength in slices where PKC had been previously activated (*, $P < 0.05$, Student's t test compared with untreated control slices).

the *persistence* of this change (and/or its functional replacement) requires additional postsynaptic messenger systems.

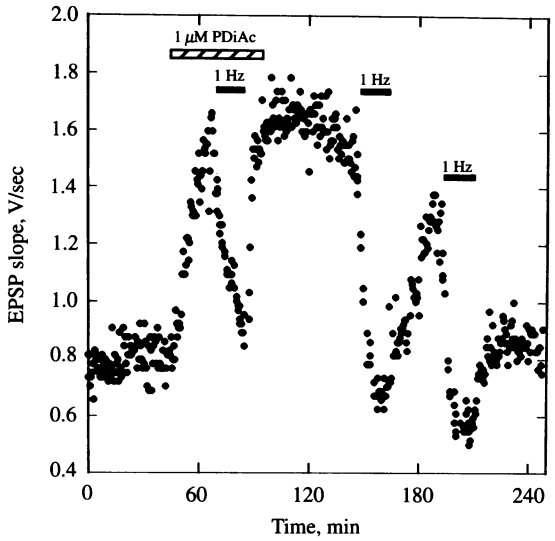

FIG. 3. LFS applied while PKC is activated does not produce enhanced LTD of synaptic strength. Plot of SCH-evoked EPSP slopes (V/sec) versus time in an experiment where PDiAc (1 μM) was bath applied, and a LFS (1 Hz × 15 min) was given *during* drug application. This LFS produced only transient depression of synaptic transmission. In contrast, the same LFS, given a second and third time after washout of PDiAc, now evoked significant LTD of synaptic strength.

In the absence of these messengers, the newly phosphorylated site would be more susceptible to dephosphorylation, leading to reversal of phorbol-induced potentiation (although complicated experimentally by relatively slow washout of lipophilic phorbol ester from the bathing medium) and a lower threshold for induction of LTD by LFS. This hypothesis is also consistent with a recent report that inhibitors of serine (threonine) protein phosphatase activity can block the induction and expression of LTD by LFS (25). However, it is quite possible that a different phosphorylation substrate of PKC, with either catalytic or second messenger properties, is the promoter of enhanced LTD.

It is less evident how reversible AMPA receptor phosphorylation could explain the PKC-induced reduction in likelihood (raised threshold) for subsequent induction of LTP by HFS. It seems clear that some protein phosphorylated by PKC either prevents the stabilization of LTP (perhaps also reducing PTP) or actively suppresses its induction. However, the identity(ies) of target protein(s) that can either enhance LTD or suppress LTP are unknown. A recent report has suggested that transient activation of NMDA receptors is also sufficient to suppress subsequent induction of LTP (14). It is not clear whether this suppression shares any cellular mechanisms with the similar effect of transient PKC activation, but it seems plausible to hypothesize that modest NMDA receptor activation might activate PKC without activating other, higher threshold, calcium-dependent mechanisms that are necessary to induce LTP.

Our previous studies (13) found that repeated induction of either short-term potentiation (STP) or LTP also lowered the threshold for LTD—that is, LFS applied after induction of either STP or LTP yielded significantly larger amplitude LTD. However, in contrast to the findings of Huang *et al.* (14) for LTP, transient activation of *N*-methyl-D-aspartate (NMDA) receptors by direct NMDA application was *not* sufficient to prime the induction of LTD. These results suggest that, while NMDA receptor activation is sufficient to suppress LTP, some additional cellular events are required to enhance LTD. Apparently all of these cellular constraints are satisfied by the transient activation of PKC.

Although the data presented here elucidate a role for PKC in regulating bidirectional long-term synaptic plasticity, they do not indicate the isozyme(s) or site(s) of action involved. It is thought that phorbol ester-induced potentiation of synaptic EPSPs may be a presynaptic phenomenon (22), but it has also been reported that the postsynaptic injection of PKC inhibitors into single pyramidal neurons can selectively prevent the induction of LTP at synapses on that neuron (18, 19). Since phorbol ester is a nonselective activator of multiple PKC isozymes, it is quite possible that different isozymes on both sides of the synapse contribute to the synaptic dialogue that establishes LTP. In any event, it is yet to be determined whether trans-synaptic communication can also play a role in the dynamic regulation of thresholds for the induction of LTP and LTD.

The dynamic regulation of the threshold for stimulation necessary to cause long-term changes in connection strength is an important computational capability that has been an essential feature of many models of neural processing systems with synaptic plasticity (6). In essence, the postulated function of such regulation is to prevent the saturation of synaptic plasticity in either a maximally potentiated or depressed state, maintaining the strength of a synapse somewhere along the linear portion of its input/output function. However, an equally important feature of this regulation is that it be linked in some way to the previous history of synaptic changes that have been induced, so that the amplitude and directional bias for long-term changes in synaptic strength are sensitive to previous position on the sliding scale. The studies presented here implicate the Ca^{2+}-PKC second messenger cascade as a component of dynamic activity-dependent regulation of synaptic plasticity.

The cellular mechanisms we have uncovered for dynamically regulating the ability to induce long-term synaptic plasticity seem likely to play important state-dependent roles in memory processing and storage. A recent *in vivo* study (26) found that synaptic activity in the frequency range of endogenous θ rhythm (5–8 Hz) doubled the amount of LTD that could be elicited at perforant path-granule cell synapses in the dentate gyrus. If factors such as the switching on and off of θ rhythm, level of arousal, and neuronal oscillations during sleep–wake cycles can cause PKC-mediated changes in threshold, this may enable them to dynamically change the gain of cellular mechanisms that produce long-term alterations in synaptic strength. One intriguing hypothesis is that a suppression of LTP and/or enhancement of LTD during rapid eye movement sleep could be necessary for sleep-related memory consolidation. Further elucidation of the cellular bases of this regulation will be an important step in understanding the role(s) synaptic plasticity plays in information processing, memory storage and retrieval.

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