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Characterization of the Integration Time for the Stabilization of Long-Term Potentiation in Area CA1 of the Hippocampus

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In area CA1 of the hippocampus, synaptic activation of NMDA receptors during postsynaptic depolarization can generate either a decremental synaptic potentiation termed short-term potentiation (STP) or stable, long-term potentiation (LTP). Examining the relationship between these two forms of synaptic enhancement should provide information about the intracellular processes responsible for the stabilization of LTP. Using the hippocampal slice preparation, initial experiments confirmed that STP can be generated either by a weak tetanus or by pairing a single EPSP with postsynaptic depolarization. Following the generation of submaximal LTP, application of a weak, STP-inducing tetanus resulted in STP (not LTP), suggesting that the processes responsible for stabilizing LTP must be activated during induction and cannot be accessed at later times. To determine the interval over which processes activated during STP can be integrated and result in stable LTP (the "integration time" for the stabilization of LTP), a fixed number of afferent stimuli were given at varying intervals (5–60 sec) during postsynaptic depolarization. Using either extracellular or whole-cell recording, LTP was rarely (11% of experiments) elicited at 1 min intervals and frequently (76% of experiments) elicited at 10 sec intervals. These results indicate that following a single EPSP during postsynaptic depolarization, the processes responsible for the stabilization of LTP decay significantly within approximately 1 min, although this value may depend on the level of activation of the requisite intracellular processes.

A defining characteristic of all forms of long-term potentiation (LTP) is that the increase in synaptic efficacy is stable over long periods of time ranging from days or weeks in the intact animal to hours in brain slice preparations. This stability over time is one important feature of LTP that distinguishes it from other, shorter forms of synaptic plasticity (Zucker, 1989). The processes responsible for the stabilization (or maintenance) of NMDA receptor–dependent LTP in the hippocampus have been the object of much investigation, leading to the development of a number of hypotheses outlining sequential biochemical steps required for the so-called "induction" and "maintenance" of LTP (for reviews, see Linden and Routtenberg, 1989; Bliss et al., 1990; Lynch et al., 1990; Malenka and Nicoll, 1990; Madison et al., 1991). Inherent in some of these models is the assumption that the processes underlying "induction" are experimentally separable from those responsible for the "stabilization" or "maintenance." The main experimental result considered consistent with this view is that in the presence of a variety of drugs presumed to act specifically on target enzymes (e.g., protein kinases, phospholipases), LTP-inducing stimuli do not elicit a long-lasting, stable potentiation of synaptic transmission but instead generate a decremental synaptic enhancement that decays to baseline within 20–40 min.

A somewhat disparate, although not irreconcilable, view is that the stabilization of LTP depends on induction conditions (Larson et al., 1986; Gustafsson and Wigström, 1990; Malenka, 1991). Consistent with this proposal are experiments demonstrating that physiological stimuli are capable of generating a decremental, short-lasting potentiation of synaptic transmission often called short-term potentiation (STP) (Racine and Milgram, 1983; Larson et al., 1986; Anwyl et al., 1989; Malenka, 1991), which, in the CA1 region of the hippocampus, is dependent on postsynaptic NMDA receptor activation (Larson and Lynch, 1988; Anwyl et al., 1989; Malenka, 1991). Recent work (Malenka, 1991) has demonstrated that conversion of decremental STP to stable LTP can be accomplished by subtle changes in induction conditions that would be expected to affect the magnitude of the NMDA receptor–mediated rise in calcium in the postsynaptic dendritic spine.

The present study was undertaken to examine further the physiological parameters that influence the stabilization of LTP. Specifically, we examine the interval over which processes activated during STP or following a single afferent stimulation (i.e., a single EPSP) during postsynaptic depolarization can be integrated and result in stable LTP. Information about this "integration time" should provide clues to the underlying biochemical mechanisms responsible for the stabilization of LTP and also may prove to be important for a detailed understanding of the role of LTP in nervous system function.

Materials and Methods

Standard methods were used to prepare hippocampal slices from Sprague–Dawley rats (2–8 weeks) (Nicoll and Alger, 1981; Malenka et al., 1988). Animals were deeply anesthetized with halothane prior to blunt dissection to remove the hippocampus. Slices (400 μm) were cut and placed in a holding chamber for at least 1 hr. A single slice was transferred to the recording chamber where it was submerged beneath a continuously superfusing solution (1.5–2.0 ml/min) that had been saturated with 95% O_2, 5% CO_2. The composition of the solution was

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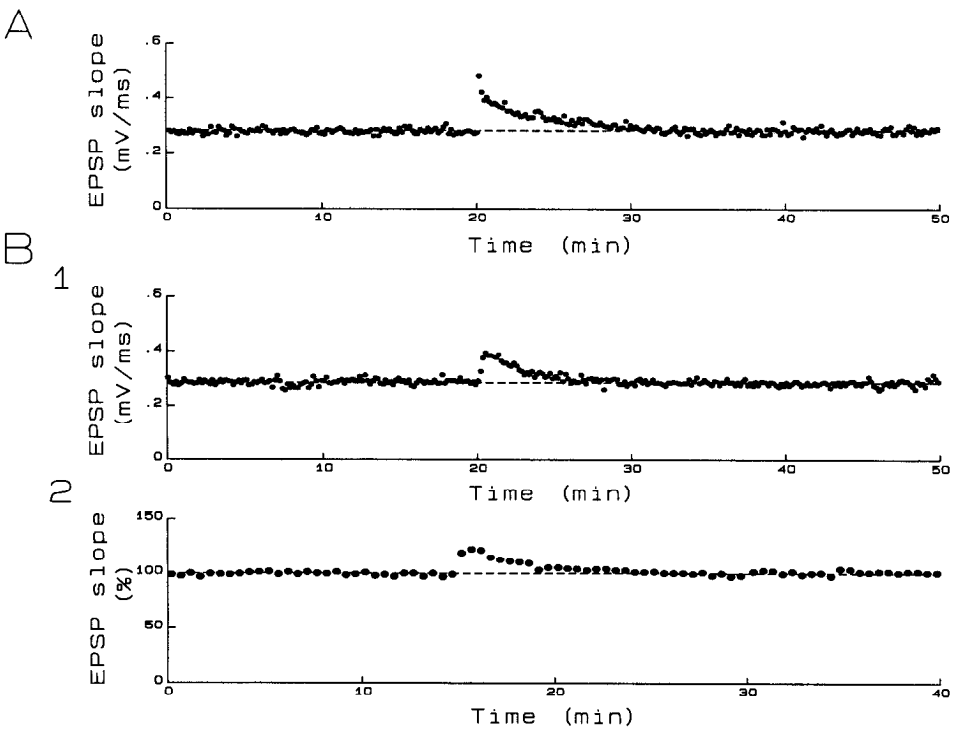

Figure 1. STP decays to baseline and is distinct from small-magnitude LTP. *A* and *B*, Graphs of the strength of synaptic transmission from experiments in which a weak tetanus (*A*; 40 Hz, 0.5 sec) or a single pairing (*B1*) was given at 20 min. In both experiments, the EPSP returned to its control value with no evidence of any stable synaptic enhancement. *B2*, Graph of the average of seven experiments in which a single pairing was performed. For over 85% of the points, the SEM is smaller than the diameter of a point.

(in mM) NaCl, 119; KCl, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; $CaCl_2$, 2.5; and glucose, 11. When picrotoxin (30–100 μM) was added to the solution, the concentration of divalent cations was increased to suppress the burst firing that often occurs in disinhibited slices ($CaCl_2$, $MgCl_2$, and KCl were all increased to 4.0 mM). The CA3 region was also surgically removed to prevent CA3 burst firing from interfering with the recordings. The temperature of the solution was maintained at 29–31°C.

Extracellular field EPSPs were recorded in stratum radiatum with electrodes (2–6 MΩ) filled with 3 M NaCl. "Blind" whole-cell patch-clamp recordings (Blanton et al., 1989; Coleman and Miller, 1989) were made from CA1 pyramidal cells with electrodes (2–6 MΩ) filled with (in mM) cesium gluconate, 117.5; CsCl, 17.5; NaCl, 8; HEPES, 10.0; EGTA, 0.2; Mg-ATP, 2; GTP, 0.2; pH 7.2. For voltage-clamp recordings, the continuous mode of an Axoclamp 2A amplifier (Axon Instruments) was used with 50–70% series resistance (8–45 MΩ) compensation. During synaptic stimulation, cells were held between −70 and −85 mV. To elicit synaptic currents, Schaffer collateral/commissural afferents in stratum radiatum were stimulated at 0.1 Hz with bipolar stainless steel electrodes. Data were collected and analyzed on line (2–10 kHz sampling rate) using a 386 IBM PC clone computer programmed with the AXOBASIC system (Axon Instruments). Initial slopes of field EPSPs were calculated using a least-squares regression. For whole-cell recording, the peak amplitude of excitatory postsynaptic currents (EPSCs) was measured.

For experiments requiring activation of two independent afferent pathways, stimulating electrodes were placed on opposite sides of the recording microelectrode. The independence of the two populations of afferents was confirmed by testing for the absence of any discernible paired-pulse facilitation (interstimulus interval, 50 msec) between the two inputs. For some experiments, STP or LTP was elicited by "pairing" low-frequency afferent stimulation with postsynaptic depolarization elicited by applying a brief tetanus (100 Hz, 50–80 msec) to a "conditioning" pathway when field EPSPs were recorded (see Gustafsson and Wigström, 1986) or by current injection into a single cell during whole-cell recording. When one pathway served as the conditioning input, before beginning the experiment repetitive tetani (100 Hz, 1 sec) were applied to potentiate synaptic transmission maximally and saturate LTP mechanisms. During pairing, the tetanus to the conditioning pathway was given 10 msec before or 5 msec after the test stimulus.

When potentiation was generated using pairing protocols, the magnitude of initial potentiation was measured immediately after the final pairing; when tetani were used, it was measured 1 min after the tetanus to avoid significant contamination by posttetanic phenomena unrelated to STP or LTP. LTP was defined as any discernable (>approximately 5%) potentiation at 30 min postinduction. The magnitude of potentiation is described in terms of percentage of the baseline EPSP or EPSC (e.g., 150% potentiation means the EPSC amplitude post-LTP is 1.5 times the baseline EPSC amplitude). Averaged graphs (see Figs. 1*B2*, 6, 8) of grouped experiments were obtained by aligning individual normalized experiments with respect to the time of the induction protocol and averaging them (Malenka et al., 1989). Each point in these graphs is the mean ± SEM. In the text, stated means are ± SD.

Results

The experiments described in this article are based on the critical assumption that is is possible to distinguish a decremental synaptic enhancement that returns to control values (STP) and the long-lasting, stable potentiation that defines LTP. An alternate possibility is that so-called STP simply represents stable LTP of such small magnitude that it is extremely difficult to distinguish the potentiation of synaptic transmission from noise in the baseline measurements. Although this possibility cannot be definitively ruled out, Figure 1 shows examples of experiments in which long, stable baselines were obtained prior to inducing STP either by a weak tetanus (40 Hz, 0.5 sec; *A*) or by pairing a single afferent stimulus with postsynaptic depolarization (a single pairing; *B1* and *B2*). Both direct inspection and statistical analysis (comparing the mean of 60 successive EPSP slope measurements obtained prior to STP generation with the mean of 60 measurements obtained 15 min after STP induction; Student's *t* test, $p > 0.10$) indicate that the EPSP decayed back to baseline and no stable, long-lasting potentiation occurred. STP can be generated repeatedly in a single pathway (Fig. 2), and its time course distinguishes it both from LTP and from the NMDA receptor–independent synaptic potentiation generated as a consequence of the presynaptic tetanus (see Zucker, 1989). An analysis of all episodes of STP elicited either by a single weak tetanus (25–50 Hz, 0.3–0.5 sec) or a single pairing reveals an initial

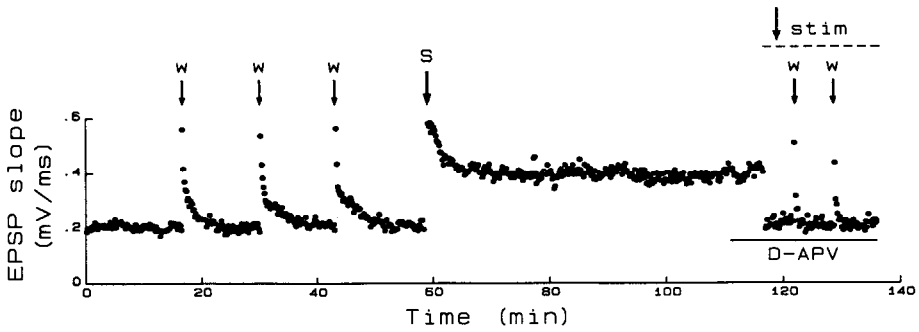

Figure 2. STP can be generated repeatedly in a single pathway and requires NMDA receptor activation. Graph of an experiment in which a weak tetanus (*w*; 30 Hz, 0.5 sec) generated STP three times in succession. A subsequent stronger tetanus (*S*; 100 Hz, 1 sec, two times, 30 sec apart) generated stable LTP. At 110 min, 25 μM D-aminophosphonovaleric acid (*D-APV*) was applied; the stimulus strength was then reduced and the same weak tetanus (*w*) applied, resulting only in a 30–40 sec synaptic potentiation.

potentiation of 128.6 ± 12.1% (n = 42), a duration of 8.6 ± 3.4 min, and a time constant of decay of 5.3 ± 2.2 min.

It has been established that the processes responsible for NMDA receptor–dependent LTP do not interact with those activated during paired-pulse facilitation (McNaughton, 1982; Muller and Lynch, 1989; Zalutsky and Nicoll, 1990). Since STP is dependent on NMDA receptor activation (Larson and Lynch, 1988; Anwyl et al., 1989; Malenka, 1991), it would be expected that STP should also not interact with paired-pulse facilitation. However, in the dentate gyrus *in vivo,* brief tetanic stimulation causes an STP-like transient synaptic potentiation during which the magnitude of paired-pulse facilitation decreases (McNaughton, 1982). Figure 3 shows an experiment (n = 3) demonstrating that, in the CA1 region, STP does not interact with paired-pulse facilitation. Following a weak tetanus (40 Hz, 0.5 sec), the magnitude of paired-pulse facilitation transiently decreased but recovered well before the decay of STP.

The experiments presented thus far further demonstrate that STP is a reproducible phenomenon that appears identical to LTP except that the synaptic enhancement is not stabilized. How do the processes activated during STP interact with those activated during LTP? Previous work has shown that saturation of LTP may transiently occlude STP-like phenomena (Kauer et al., 1988; Gustafsson et al., 1989). However, a distinct question is whether the processes activated during STP can sum with those responsible for maintaining LTP, as might be expected if the processes responsible for LTP induction and maintenance are separable. Figure 4 shows examples of experiments (n = 8) in which a weak STP-inducing tetanus was given before and during stable LTP. When applied during stable LTP, the weak tetanus still was capable of eliciting only STP. This was observed even when stimulus strength was not changed (Fig. 4*B*) such that the EPSP was larger during the second weak tetanus. The inability to generate LTP with the second weak tetanus was not due to saturation of LTP since a subsequent stronger tetanus caused further stable LTP.

The results of the previous experiment suggest that the processes responsible for the stabilization of LTP must be activated during LTP induction and cannot be accessed at later times. These processes must also be activated to a certain degree during STP since pairing low-frequency afferent stimulation with postsynaptic depolarization can generate robust, stable LTP (Gustafsson and Wigström, 1986; Kelso et al., 1986; Sastry et al., 1986). It was therefore of interest to determine the time interval

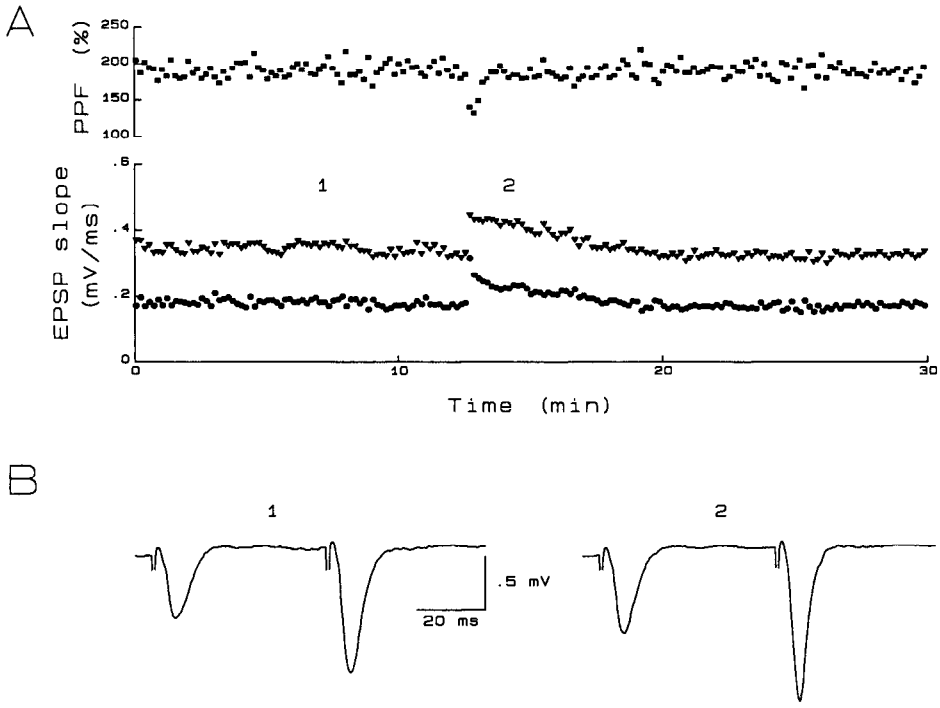

Figure 3. Paired-pulse facilitation is not affected by STP. *A,* Graphs from an experiment in which paired-pulse facilitation was monitored continuously at a 50 msec interpulse interval. The *bottom graphs* plot the EPSP slope of the responses to the first (●) and second (▼) stimuli. The *top graph* plots the magnitude of the paired-pulse facilitation (*PPF*; 200% indicates that the EPSP slope of the second response was twice that of the first). Note that the magnitude of paired-pulse facilitation decreased for only approximately 50–60 sec while STP lasted 6–8 min. *B,* Sample data traces (average of three successive sweeps) taken at the time indicated by the *numbers* in *A.*

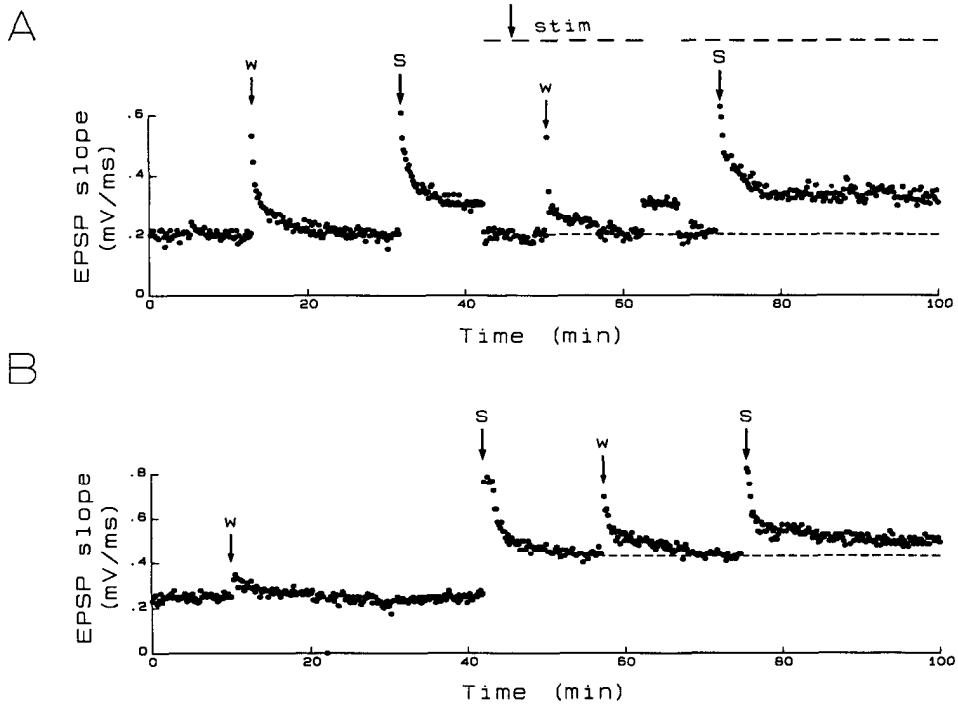

Figure 4. The processes activated during STP cannot sum with the processes responsible for maintaining LTP. *A*, Graph of an experiment in which a weak (*w*; 40 Hz, 0.5 sec) tetanus initially elicited STP and a subsequent strong (*S*; 100 Hz, 1 sec) tetanus generated LTP. Stimulus strength was then reduced to elicit an EPSP comparable to EPSPs recorded during the baseline period and the same weak tetanus (*w*) applied, resulting again in STP. Stimulus strength was then returned to its original value, demonstrating that LTP had not decayed. When the strong tetanus (*S*) was again applied at the lower stimulus strength, LTP was generated, indicating that LTP mechanisms had not been saturated. *B*, Graph of an experiment like that in *A* except the second weak tetanus was applied without changing stimulus strength. Despite the larger EPSP, the weak tetanus generated STP, not LTP. As in *A*, this was not due to saturation of LTP mechanisms since a subsequent strong tetanus generated further stable LTP.

over which the processes activated during STP could be integrated and result in stable LTP (this interval will be termed the "integration time"). We found that applying a weak STP-inducing tetanus two to four times at 40–60 sec intervals rarely elicited LTP (17% of slices; $n = 12$) even though the later tetani were often applied when the EPSP was significantly potentiated.

Although the previous experiments using weak tetani provided useful preliminary information, the magnitude of STP induced by these repetitive weak tetani was quite variable (initial potentiation ranged from 120% to 230%). We therefore focused on determining the "integration time" following activation of NMDA receptor–dependent processes by single EPSPs. In one set of experiments, this was accomplished by examining the effects of changing the interval between single pairings using a brief tetanus to a conditioning pathway to provide postsynaptic depolarization. Figure 5 shows an experiment in which 10 pairings at a 30 sec interval resulted in STP whereas 10 pairings at a 10 sec interval resulted in stable LTP (Fig. 5*A*, top graph). The lower graph shows that the size of the EPSP in the conditioning pathway did not change (and in fact slightly decreased) during the experiment, making it unlikely that the magnitude of the induced postsynaptic depolarization (see Malenka, 1991) differed significantly when the pairings were performed (although it is possible that the conditioning EPSP was transiently increased during the 10 sec pairings because of short-lasting, posttetanic synaptic potentiation).

Figure 6 shows a summary of these experiments. When the interval between pairings was 1 min (Fig. 6*A*), the potentiation

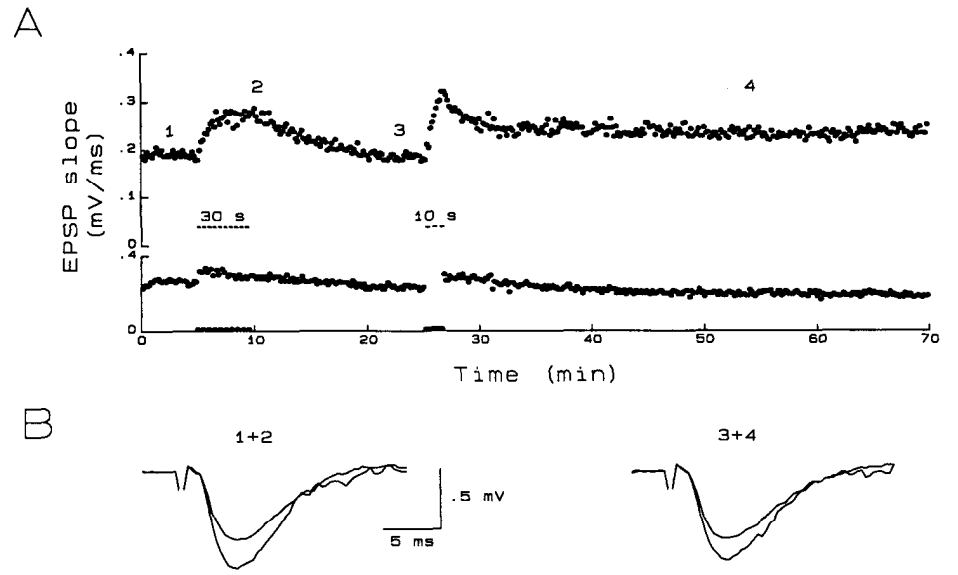

Figure 5. The stabilization of LTP depends on the interval between successive pairings using a conditioning pathway to elicit postsynaptic depolarization. *A*, Graphs of an experiment in which 10 pairings were performed, first at a 30 sec interval (*30 s*) and then at a 10 sec interval (*10 s*). The *top graph* plots the test pathway EPSP slope; the *bottom graph* plots the conditioning pathway EPSP slope. The conditioning stimulation was 100 Hz, 50 msec given 5 msec after the test stimulation. *B*, Superimposed data traces taken at the times indicated by the *numbers* in *A*.

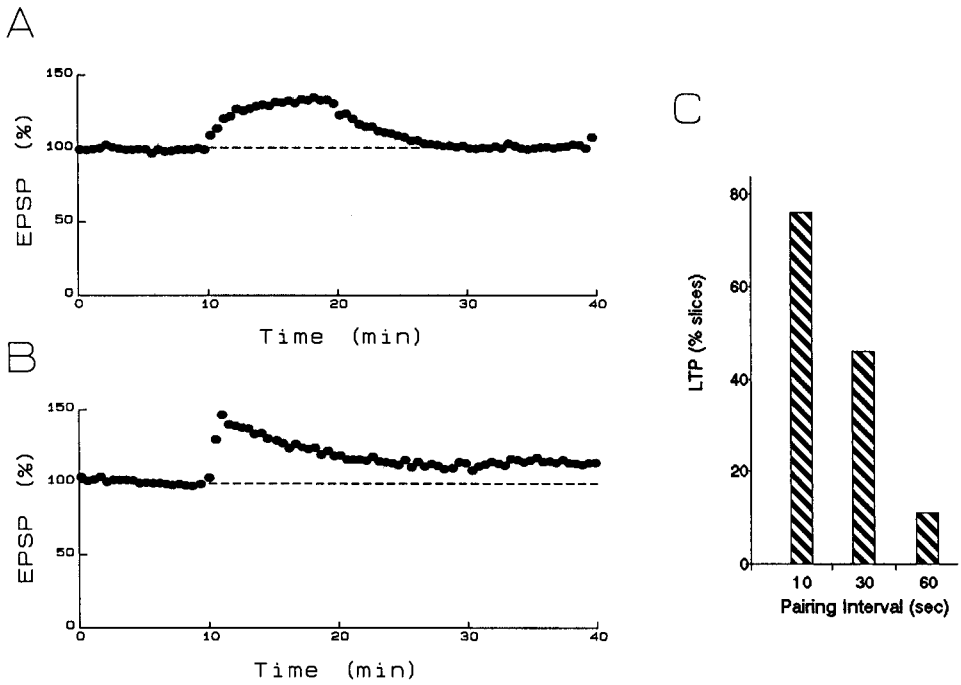

Figure 6. Summary of the extracellular pairing experiments. *A* and *B*, Averaged graphs of experiments in which 10 pairings were performed at 1 min (*A*; $n = 7$) or 10 sec (*B*; $n = 9$) intervals. In each graph, for over 75% of the points, the SEM is smaller than the diameter of a point. *C*, Graph showing the percentage of experiments resulting in LTP as a function of the interval between pairings (*10 sec*, $n = 21$; *30 sec*, $n = 11$; *60 sec*, $n = 9$).

was 137.4 ± 11.0% ($n = 9$) with a duration of 10.6 ± 5.7 min and a time constant of decay of 4.7 ± 3.4 min. Shortening the interval to 10 sec (Fig. 6*B*) increased the magnitude of the potentiation to 152.8 ± 17.9% ($n = 17$), which at 30 min decayed to a stable value of 111.4 ± 7.5% (this value includes those experiments in which LTP was not elicited). Figure 6*C* shows the percentage of experiments resulting in LTP as a function of the interval between pairings. It is clear that LTP became progressively easier to generate as the interval between pairings decreased from 1 min to 10 sec.

Using whole-cell recording, it was possible to control more precisely the level of postsynaptic depolarization during the pairing event. To compare the effects of varying the interval between pairings in a single cell, two independent inputs were alternately stimulated. After a 10–15 min baseline was obtained, the cell was depolarized to −20 to −35 mV and one pathway was stimulated every 30–60 sec while the other pathway received the same number of stimuli every 10 sec. Because the ability to generate LTP may "wash out" during whole-cell recording (Malinow and Tsien, 1990), the shorter-interval stimuli

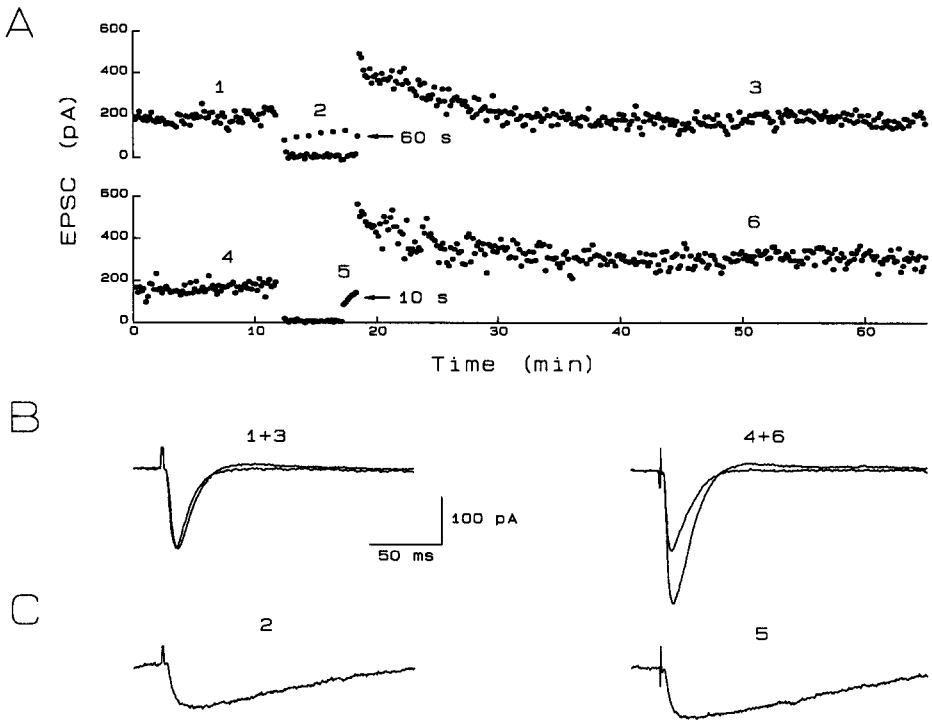

Figure 7. The stabilization of LTP in a single cell depends on the interval between successive pairings. *A*, Graphs of an experiment in which two pathways were alternately stimulated. At 12 min, the cell was depolarized (−30 mV), and then one pathway (*top graph*) was stimulated every 60 sec, resulting in STP. The other pathway (*bottom graph*) received the same number of stimuli (seven) every 10 sec at the end of the pairing period, resulting in LTP. *B*, Superimposed data traces taken at the times indicated by the *numbers* in *A*. Each trace is the average of three successive sweeps. *C*, Traces recorded during depolarization to −30 mV.

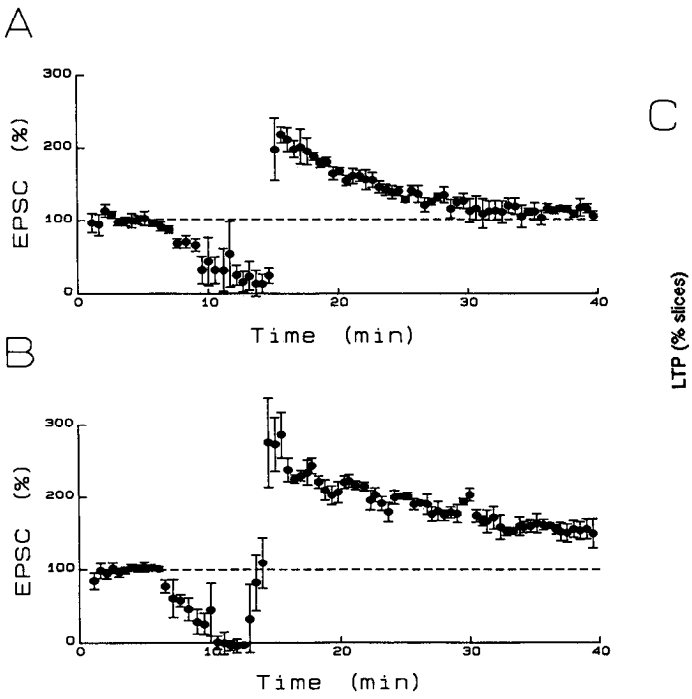

Figure 8. Summary of the whole-cell experiments. *A* and *B*, Averaged graphs of experiments in which stimuli were given during depolarization at 40–60 sec (*A*; $n = 9$) or 10 sec (*B*; $n = 9$) intervals. The individual experiments averaged to construct these graphs were taken from the same nine cells. The slow decay and increased scatter at 5 min is because the number of stimuli given during depolarization varied from 6 to 10. Individual experiments were aligned with respect to the time at the end of the pairings when the cells were returned to control membrane potential. *C*, Graph showing percentage of experiments resulting in stable LTP as a function of the interval between stimuli during depolarization (*10 sec*, $n = 16$; *30 sec*, $n = 7$; *40–60 sec*, $n = 9$).

were given at the end of the long-interval stimuli. Figure 7 shows an example of this experiment. Depolarizing the cell evoked an EPSC with a prominent NMDA receptor–mediated component (Fig. 7*C*; Hestrin et al., 1990). STP was elicited in the pathway stimulated seven times with a 60 sec interstimulus interval (Fig. 7*A*, top graph), while stable LTP was generated in the pathway activated every 10 sec (bottom graph). Control experiments ($n = 3$) demonstrated that stopping afferent stimulation for 5–10 min had no long-lasting (>5 min) effects on the strength of synaptic transmission).

Figure 8 is a summary of the whole-cell experiments. When the interval between pairings was 40–60 sec (Fig. 8*A*), STP was elicited, while stimulating every 10 sec during depolarization generated stable LTP (Fig. 8*B*). Figure 8*C* shows that the percentage of experiments resulting in LTP using whole-cell recording was remarkably similar to the values obtained with extracellular recording. The only conspicuous difference between the two sets of experiments was in the magnitude of the initial potentiation (see Discussion).

Discussion

The biochemical processes responsible for the stabilization of LTP have been the subject of intense investigation, with most experiments using pharmacological probes to distinguish the processes responsible for the "induction" of LTP from those responsible for its "maintenance." Because surprisingly little is known about the physiological parameters that influence the stabilization of LTP and because such information may provide important clues to the underlying biochemical mechanisms, we have performed experiments examining the relationship between two NMDA receptor–dependent forms of synaptic enhancement, STP and LTP.

In the initial experiments presented here we confirmed that, given the resolution of recording techniques, STP shares many properties with LTP, the only notable difference being that the initial potentiation is transient and decays back to control values. Consistent with the proposal that the processes responsible for the stabilization of LTP must be activated during LTP induction was the finding that following establishment of submaximal stable LTP, an STP-inducing stimulus still only generated STP. Previous results also demonstrate a strong link between induction conditions and the stabilization of LTP (Larson et al., 1986; Gustafsson and Wigström, 1990; Malenka, 1991). Thus, it may be quite difficult to separate experimentally the processes of LTP induction from those responsible for maintenance.

Pairing a single afferent stimulus with postsynaptic depolarization routinely resulted in STP (Fig. 1*B*), yet a number of such pairings result in stable LTP (Gustafsson and Wigström, 1986; Kelso et al., 1986; Sastry et al., 1986). This indicates that there must be some interval over which the processes activated by a single EPSP can be integrated. Our results using two different techniques to pair synaptic stimulation with postsynaptic depolarization suggest that for the processes responsible for LTP stabilization, this "integration time" is on the order of 1 min. Since LTP likely depends on a rise in postsynaptic calcium (Lynch et al., 1983; Malenka et al., 1988) and imaging studies in brain slices indicate that increases in calcium due to synaptic activation of NMDA receptors decay significantly within at most 3–4 sec (Reghr and Tank, 1990; Yuste and Katz, 1991), it is likely that the integration time reflects the decay of calcium-activated processes rather than the decay of calcium itself. To our knowledge, the only other study performing similar experiments (Skelton et al., 1983) found that in the perforant path–dentate gyrus system *in vivo*, applying a fixed number of single strong stimuli caused a long-lasting (>1 week) potentiation of synaptic transmission when stimuli were given every 5–10 sec but not when applied every 25 sec. It is striking that using a completely different experimental paradigm, the stable expression of LTP was demonstrated to be sensitive to transient anoxia for a period of 1–2 min following LTP induction (Arai et al., 1990).

Although changing the interval between successive pairings elicited similar results with both extracellular and whole-cell recording, the magnitude of initial potentiation was larger when the cell was directly depolarized during whole-cell recording. This difference was also observed in some of the original studies of pairing-induced LTP using extracellular and intracellular recording (see Gustafsson and Wigström, 1986; Gustafsson et al., 1987). This difference may reflect the magnitude of potentiation at individual synapses or the fraction of sampled synapses significantly potentiated by the pairing protocol. The consistency in the integration time, despite differences in the initial potentiation, would suggest that the latter explanation is more likely and therefore that the integration time reflects the processes occurring at a single synaptic site.

A previous study (Malenka, 1991) on STP and LTP demonstrated that the magnitude of calcium influx through the NMDA receptor ionophore may be an important variable influencing the duration of synaptic potentiation. Assuming that the stabilization of LTP requires activation of a threshold concentration of some calcium-dependent factor, the "integration time" will also be dependent on the magnitude of the initial rise in calcium (i.e., the induction conditions) since larger increases in postsynaptic calcium would be expected to prolong the decay of calcium-dependent processes (see Zador et al., 1990). That is, an increase in the initial level of activity of a presumptive calcium-activated factor would prolong the interval over which this activity could be integrated, assuming that the decay time constant of this activity remained fixed. The integration time, however, also will depend critically on the rate of decay of the activated process, a parameter that itself may be under independent control. For example, if a rise in calcium results in activation of CaMKII, the duration of auto- and substrate phosphorylation will be strongly influenced not only by the magnitude of the initial rise in calcium but also by the rate and magnitude of phosphatase activity (see Lisman, 1989).

Although the presented experiments have not addressed the actual biochemical processes mediating the stabilization of LTP, they indicate that these processes must be activated during LTP induction and that following a single EPSP, they decay significantly within approximately 1 min. Assuming that an EPSP at a single synapse is incapable of providing sufficient depolarization to induce LTP, the limit on the integration time suggests that several synapses must be activated simultaneously and repetitively within this temporal window to generate LTP. Such constraints may ensure that only "meaningful" correlated pre- and postsynaptic activity results in stable, long-lasting synaptic modifications.

References

Anwyl R, Mulkeen D, Rowan MJ (1989) The role of *N*-methyl-D-aspartate receptors in the generation of short-term potentiation in the rat hippocampus. Brain Res 503:148–151.

Arai A, Larson J, Lynch G (1990) Anoxia reveals a vulnerable period in the development of long-term potentiation. Brain Res 511:353–357.

Blanton M, LoTurco J, Kriegstein A (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J Neurosci Methods 30:203–210.

Bliss TVP, Clements MP, Errington ML, Lynch MA, Williams JH (1990) Presynaptic changes associated with long-term potentiation in the dentate gyrus. Semin Neurosci 2:345–354.

Coleman PA, Miller RF (1989) Measurement of passive membrane properties with whole-cell recordings from neurons in the intact amphibian retina. J Neurophysiol 61:218–230.

Gustafsson B, Wigström H (1986) Hippocampal long-lasting potentiation produced by pairing single volleys and brief conditioning tetani evoked in separate afferents. J Neurosci 6:1575–1582.

Gustafsson B, Wigström H (1990) Basic features of long-term potentiation in the hippocampus. Semin Neurosci 2:321–334.

Gustafsson B, Wigström H, Abraham WC, Huang Y-Y (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J Neurosci 7:774–780.

Gustafsson B, Asztely F, Hanse E, Wigström H (1989) Onset characteristics of long-term potentiation in the guinea-pig hippocampal CA1 region *in vitro*. Eur J Neurosci 1:382–394.

Hestrin S, Nicoll RA, Perkel DJ, Sah P (1990) Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. J Physiol (Lond) 422:203–225.

Kauer JA, Malenka RC, Nicoll RA (1988) NMDA application potentiates synaptic transmission in the hippocampus. Nature 334:250–252.

Kelso SR, Ganong AH, Brown TH (1986) Hebbian synapses in hippocampus. Proc Natl Acad Sci USA 83:5326–5330.

Larson J, Lynch G (1988) Role of *N*-methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal θ-rhythm. Brain Res 441:111–118.

Larson J, Wong D, Lynch G (1986) Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res 368:347–350.

Linden DJ, Routtenberg A (1989) The role of protein kinase C in long-term potentiation: a testable model. Brain Res Rev 14:279–296.

Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. Proc Natl Acad Sci USA 86: 9574–9578.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305:719–721.

Lynch G, Kessler M, Arai A, Larson J (1990) The nature and causes of hippocampal long-term potentiation. Prog Brain Res 83:233–250.

Madison DV, Malenka RC, Nicoll RA (1991) Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 14:379–397.

Malenka RC (1991) Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. Neuron 6:53–60.

Malenka RC, Nicoll RA (1990) Intracellular signals and LTP. Semin Neurosci 2:335–344.

Malenka RC, Kauer JA, Zucker RJ, Nicoll RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81–84.

Malenka RC, Kauer JA, Perkel DJ, Mauk MD, Kelly PT, Nicoll RA, Waxham MN (1989) An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340: 554–557.

Malinow R, Tsien RW (1990) Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. Nature 346:177–180.

McNaughton BL (1982) Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. J Physiol (Lond) 324:249–262.

Muller D, Lynch G (1989) Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in hippocampus. Brain Res 479:290–299.

Nicoll RA, Alger BE (1981) A simple chamber for recording from submerged brain slices. J Neurosci Methods 4:153–156.

Racine RJ, Milgram NW (1983) Short-term potentiation phenomena in the rat limbic forebrain. Brain Res 260:201–216.

Reghr WG, Tank DW (1990) Postsynaptic NMDA receptor–mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. Nature 345:807–810.

Sastry BR, Goh JW, Auyeung A (1986) Associative induction of post-tetanic and long-term potentiation in CA1 neurons of rat hippocampus. Science 232:988–990.

Skelton RW, Miller JJ, Phillips AG (1983) Low-frequency stimulation of the perforant path produces long-term potentiation in the dentate gyrus of unanesthetized rats. Can J Physiol Pharmacol 61:1156–1161.

Yuste R, Katz LC (1991) Control of postsynaptic Ca^{2+} influx in de-

veloping neocortex by excitatory and inhibitory neurotransmitters. Neuron 6:333–344.
Zador A, Koch C, Brown TH (1990) Biophysical model of a Hebbian synapse. Proc Natl Acad Sci USA 87:6718–6722.
Zalutsky R, Nicoll RA (1990) Comparison of two forms of long-term potentiation in single hippocampal neurons. Science 248:1619–1624.
Zucker RS (1989) Short-term synaptic plasticity. Annu Rev Neurosci 12:13–31.