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Proactive and Retrograde Effects on LTP Produced by Theta Pulse Stimulation: Mechanisms and Characteristics of LTP Reversal in Vitro

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Abstract

Previous studies have established that (1) a 1-min episode of theta pulse stimulation (TPS) is sufficient to reverse potentiation during the early phases of LTP in area CA1 without causing depression when administered to nonpotentiated pathways; (2) the magnitude of depotentiation is inversely related to the delay between LTP induction and reversal attempts; and (3) pharmacological facilitation of AMPA receptor-mediated currents significantly enhances the strength of the reversal mechanism. The present experiments confirm and extend these results by showing that the depotentiating action of TPS on prior LTP is antagonized by inhibitors of protein phosphatases and adenosine A₁ receptors but is not affected by NMDA receptor blockade, and, moreover, that TPS interferes with subsequent LTP induction by triggering an inhibitory mechanism that is active for a few minutes and is blocked by phosphatase inhibition. The possible implications of these results are discussed.

Introduction

Previous work has shown that long-term potentiation (LTP) produced by a stimulation pattern that mimics bursting activity observed in behaving animals is best reversed by a second, natu-

rally occurring stimulation code temporally coupled with LTP induction. Specifically, experiments conducted in area CA1 revealed that brief episodes of single-pulse stimulation patterned at the frequency of the theta rhythm, if delivered shortly after LTP induction, are sufficient to reverse completely the potentiation produced by theta burst stimulation (TBS) in vitro and in freely moving animals (Staubli and Lynch 1990; Larson et al. 1993). Subsequent in vitro work provided a detailed description of the time dependence that governs the LTP reversal effect (Staubli and Chun 1996). Specifically, it was found that a 1-min train of theta pulse stimulation (TPS) almost completely reversed LTP if delivered 30 sec after induction, but had decreasing impact when given at longer intervals; that is, significant depotentiation was seen with reversal attempts conducted between 30 sec and 15 min but not at delays of 30 min and longer. TPS had no impact when given to naive pathways, and increasing the duration of TPS did not produce a greater reversal of potentiated inputs.

Our earlier work also demonstrated that pharmacological facilitation of glutamatergic transmission with the allosteric AMPA receptor modulator BDP-12, an analog of 1-(1,3-benzodioxol-5-ylcarbonyl) piperidine that produces changes in AMPA receptor kinetics (Arai et al. 1994, 1996a,b), significantly enhanced the strength of the reversal process and allowed TPS to produce robust depotentiation up to 30 min after LTP induction. The amount of depotentiation observed in the presence of the drug was dose dependent, synapse specific, reversible, and independent of NMDA receptor activation.

The goal of the present experiments was to further characterize the LTP reversal phenomenon

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and thereby gain insight into the molecular mechanisms that might contribute to depotentiation. This involved examining the relationship between variable TPS durations and reversal amount as well as determining the lower limits necessary to obtain the effect. The ability of TPS to inhibit induction of subsequent LTP was then characterized, followed by tests that assessed the contributions of protein phosphatases, NMDA receptors, and adenosine A₁ receptors to the proactive and retrograde interactions observed between LTP and TPS. Finally, it was determined whether the machinery underlying LTP reversal by TPS was different from that responsible for depotentiation obtained with a more extended low-frequency protocol (LFS) commonly used to produce long-term depression (LTD) (see Dudek and Bear 1992).

Materials and Methods

Experiments were conducted on hippocampal slices prepared from male Sprague–Dawley rats weighing 180–360 grams. The rats were decapitated, and their brains were rapidly removed and placed in 0°C oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl, 124; KCl, 3; KH₂PO₄, 1.25; MgSO₄, 2.5; CaCl₂, 3.4; NaHCO₃, 26; D-glucose, 10; and L-ascorbate, 2. One of the hippocampi was quickly dissected free in ice-cold aCSF, placed on a McIlwain tissue chopper where its middle portion was cut into 400-μm sections and collected into a petri dish containing ice-cold aCSF. From there, selected slices were placed immediately on a nylon net in an interface chamber and maintained at a temperature of 31°C ± 1°C. The slices were perfused continuously with aCSF at a rate of 75 ml/hr while their upper surface was exposed to warm humidified 95% O₂/5% CO₂. Recording began after an incubation time of 1 hr.

Population excitatory postsynaptic potentials (field EPSPs) were measured with a glass micropipette filled with 2 mM NaCl and positioned in the middle of stratum radiatum of field CA1b. Two bipolar stimulating electrodes [twisted strands of nichrome wire (65 μm)] were placed in equivalent positions of CA1a and CA1c, respectively, to activate separate (nonoverlapping) populations of Schaffer collateral/commissural (S-C) fibers. Their positions were arranged so that the same amount of current evoked two responses that did not differ from each other by >10%. The stimulus strength

of the test pulses was adjusted to produce a response whose amplitude was ~60% of the maximum spike-free response. Stimulation pulses were alternated between the two pathways at intervals of 10–20 sec, such that each pathway received a pulse every 20–40 sec. The recording signals were digitized by a computer that calculated the slope and amplitude on-line and stored the data on disk. If slope and amplitude of the field EPSPs did not fluctuate by >10% during a 15- to 20-min control period, the responses were used as baseline values before experimental tests. LTP was always induced using TBS consisting of ten 30-msec-long bursts with four pulses at 100 Hz each, separated by 200 msec, a pattern shown to be optimal for producing saturated levels of LTP (Larson et al. 1986). The stimulation intensity during TBS was increased so as to trigger a spike component in the dendritic field potential, usually by doubling the pulse duration, but remained unchanged in experiments involving simultaneous LTP induction in both pathways. Testing with single pulses was then resumed to confirm that LTP had been established. Attempts to reverse LTP were conducted with TPS consisting of single-pulse stimulation with the pulses separated by 200 msec. In one set of experiments, LFS consisting of a 15-min train at 1 Hz was applied. The stimulation intensity was not increased during TPS or LFS.

Various experiments involved bath applications of one of the following drugs: selective A₁ adenosine receptor antagonist CPX (8-cyclopentyl-1,3-dipropylxanthine) at 200 nM (RBI), NMDA receptor antagonist DL-AP5 at 200 μM (RBI), protein phosphatase inhibitor okadaic acid in 0.1–0.2% DMSO at 1.0 μM (RBI), and allosteric AMPA receptor modulator BDP-12 at 500 μM (Cortex Pharmaceuticals, Inc., Irvine, CA). Care was taken to keep the total rate of infusion into the chamber constant throughout the experiment (75 ml/hr) by adjusting the flow rate of the primary source of aCSF accordingly.

Results

Our previous study demonstrated that a 1-min train of TPS delivered 30 sec after LTP induction causes an almost complete reversal of potentiation but had increasingly less impact when administered at longer delays and virtually no effect at 30 min (Staubli and Chun 1996). Because it had never been examined whether less stimulation de-

livered at 30 sec also produces depotentiation and, if so, to what extent, the first step was to determine what the lower limits are to achieve significant depotentiation. To test this, LTP was always induced simultaneously in both pathways, TPS was delivered 30 sec later to the test input, and recording was resumed for another 50 min. Consistent with our earlier results, a near complete LTP reversal was obtained with a 1-min episode of TPS; that is, test responses were increased to $109.5 \pm 3.1\%$ of baseline at 50 min, whereas LTP in the control input was at $157.3 \pm 2.0\%$ [paired $t(4) = 12.49$, $P < 0.0001$; Fig. 1A]. Shorter TPS episodes lasting 30 or 15 sec produced progressively less depotentiation. That is, 30 sec of TPS reduced LTP in the test input to $130 \pm 4.9\%$ of baseline at 50 min, whereas control LTP levels were $176.4 \pm 6.3\%$ [paired $t(4) = 12.65$, $P < 0.0001$; Fig. 1B], and 15 sec of TPS reduced LTP to $154.1 \pm 8.9\%$ in the test input compared with $173.3 \pm 7.8\%$ of control LTP [paired $t(6) = 2.67$, $P < 0.02$; Fig. 1C]. The percent reversal (i.e., % decrease in LTP 50 min after TPS for S1 relative to S2) produced by 15 sec of TPS was $25.6 \pm 8.5\%$, significantly smaller compared with the $61.2 \pm 4.0\%$ depotentiation obtained with 30 sec of TPS [unpaired

$t(10) = 3.31$, $P < 0.005$], which in turn was significantly reduced compared with the $83.3 \pm 5.6\%$ reversal obtained with a 1-min TPS episode [unpaired $t(8) = 3.22$, $P < 0.01$].

Second, the reported ability of TPS to suppress the initiation of LTP when delivered immediately before TBS was examined (O'Dell and Kandel 1994). The objective was to first confirm the effect and then to estimate the time course of the inhibitory process by successively increasing the delay between TPS and subsequent TBS and measuring its impact on the magnitude of the resulting potentiation. When delivered to naive pathways, TPS had little effect on baseline synaptic transmission and, at most, produced a small but transient depression (Staubli and Chun 1996; Fig. 2D). However, a 1-min TPS episode delivered immediately prior to TBS prevented subsequent LTP induction (Fig. 2A). Mean test responses were $105.6 \pm 3.2\%$ of baseline 30 min after the TPS–TBS sequence, whereas the amount of LTP simultaneously induced in the control pathway was $168.0 \pm 4.5\%$. The effects of TPS were reversible; that is, a second TBS treatment 30 min afterward produced substantial LTP that was $152.4 \pm 6.9\%$ of baseline 50 min later. The ability of TPS to sup-

Figure 1: Effect of fractional TPS on depotentiation. (A) Shown are group data of experiments in which LTP was induced simultaneously in the control and the test pathways using TBS. Reversal attempts involved a 60-sec train of TPS delivered 30 sec after LTP induction to the test pathway. (Insets) Superimposed EPSPs taken at the times indicated in the graph (calibration: 1 mV/5 msec). (B,C) Same as in A except that the duration of TPS was 30 sec (B) and 15 sec (C).

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Figure 2: TPS delivered before induction of potentiation suppresses subsequent LTP, an effect that is inversely related in magnitude to the length of the TPS–TBS interval. (A) Group data of experiments in which a 60-sec train of TPS delivered to the test pathway immediately prior to TBS caused an almost complete suppression of LTP, whereas LTP induced simultaneously in the control input developed normally [paired $t(4)=10.04$, $P<0.0001$]. A second episode of TBS administered to the test input 30 min later elicited stable LTP. (B) Increasing the delay between TPS and TBS to 1 min also resulted in complete suppression of LTP in the test pathway [paired $t(4)=3.89$, $P<0.01$]. (C) TPS given 2.5 min before TBS produced less but still significant LTP suppression ([paired $t(7)=5.21$, $P<0.001$]. The magnitude of potentiation produced with a 2.5-min TPS–TBS interval was significantly larger than that obtained with a 1-min [$t(11)\leq 4.28$, $P<0.001$] or zero interval [$t(11)=3.01$, $P<0.01$]. (D) TPS given 5 min before TBS failed to suppress LTP ($n=6$).

press LTP was also present when TBS was delayed by 1 min. Test EPSPs were $101.4\pm 2.8\%$ of baseline 50 min following TBS, whereas control LTP was $150.0\pm 0.9\%$ (Fig. 2B). When TBS was given 2.5 min after TPS, LTP formation was still significantly inhibited, but to a lesser degree. Specifically, test responses were $117.5\pm 2.4\%$ of baseline 50 min following TBS, compared with $150.6\pm 4.4\%$ of control LTP (Fig. 2C). TPS failed to suppress LTP when TBS was delayed by 5 min (Fig. 2D). LTP in the test input was $150.5\pm 4.0\%$ of baseline, virtually identical to the amount of potentiation in the control input ($154.5\pm 3.3\%$).

The relatively short time course during which TPS given before TBS is able to suppress LTP and TPS delivered after TBS is effective at reversing LTP might help delineate possible cellular mechanisms that contribute to these effects. Potential candidates include activation of protein phosphatases (O'Dell and Kandel 1994) and adenosine A_1 receptors (Larson et al. 1993). Phosphatase in-

hibitors have been reported to prevent a 3-min TPS episode from blocking subsequent LTP and from depotentiating already established LTP (O'Dell and Kandel 1994), results taken to suggest that TPS activates protein phosphatases and thereby opposes the action of protein kinases that appear to be required in the early stages after TBS for the formation of stable LTP (see Bliss and Collingridge 1993). We found that the phosphatase inhibitor okadaic acid (OA) prevented TPS from producing the degree of reversal normally obtained with this paradigm (Fig. 3A). The percent reversal in the presence of OA was $35.9\pm 8.2\%$, whereas the percent decrease in test LTP relative to control LTP in our earlier experiments conducted without OA was significantly larger, as depicted in the inset of Figure 3A.

OA ($1\ \mu\text{M}$) also diminished the ability of TPS to suppress the subsequent induction of LTP (Fig. 3B). The degree of LTP blockade (% decrease in LTP 50 min after TBS for S1 relative to S2) in the

Figure 3: The phosphatase inhibitor OA suppresses depotentiation produced by TPS. (A) Group data of experiments in which LTP was induced simultaneously in test (S1) and control (S2) input in presence of OA. OA was applied at $1\ \mu\text{M}$ starting 20 min before LTP induction and was washed out after TPS. A 60-sec train of TPS delivered to S1 30 sec after LTP induction produced only partial but still significant depotentiation of the test response relative to LTP in S2 [paired $t(5) = 5.24$, $P < 0.01$]. That is, LTP in the control input was $174.3 \pm 11.4\%$ of baseline 50 min after TBS, whereas the test response in the same slices was $152.2 \pm 9.0\%$. For comparison purposes the graph also includes group data adapted from Fig. 1A, showing the amount of depotentiation obtained with this paradigm when TPS was delivered in absence of OA (S^*). (Inset) Percent reversal obtained in absence vs. presence of OA, with TPS applied immediately after TBS [nonpaired t -test: $t(9) = 4.59$; $P < 0.001$]. Waveforms above graph illustrate superimposed EPSPs taken at the times indicated (calibration: $1\ \text{mV}/5\ \text{msec}$). (B) Group data conducted in presence of OA, with TPS administered to the test pathway (S1) immediately prior to delivering TBS to S1 and S2. OA was applied at $1\ \mu\text{M}$ starting 20 min before LTP induction and was washed out after TBS. TPS caused a significant reduction of potentiation in the test input compared with LTP in S2 [paired $t(4) = 2.48$, $P < 0.03$]. Specifically, test responses were $131.6 \pm 4.9\%$ of baseline at 50 min, whereas LTP in the simultaneously tetanized control input was $164 \pm 9.5\%$. For comparison purposes the graph also includes group data adapted from Fig. 2B, showing the amount of depotentiation obtained with this paradigm when TPS was delivered in the absence of OA (S^*). (Inset) Percent reversal obtained in absence vs. presence of OA, with TPS applied immediately before TBS [nonpaired t -test: $t(8) = 6.31$, $P < 0.001$]. (C) Group data ($n = 4$) conducted in the presence of OA and AMPA receptor modulator BDP-12, with TPS administered to the test pathway (S1) 15 min after delivering TBS to S1 and S2. No reversal was obtained in the presence of OA (OA at $1\ \mu\text{M}$ and BDP-12 at $500\ \mu\text{M}$ were washed-in 15 min before LTP induction, and wash-out began immediately after TPS). For comparison purposes the graph also includes group data adapted from Staubli and Chun (1996), showing the amount of LTP suppression produced by TPS in slices perfused with BDP-12 alone ($500\ \mu\text{M}$, $n = 5$). (Inset) Compares the percent reversal obtained with BDP-12 in presence vs. absence of OA [unpaired $t(10) = 11.87$, $P < 0.0001$].

presence of OA was $44.7 \pm 5.7\%$, significantly smaller compared with the average LTP suppression produced by TPS in our earlier experiments that did not involve OA, as illustrated in the inset of Figure 3B. A 20-min bath application of 0.1% – 0.2% DMSO alone had no obvious effect on base-

line synaptic transmission and did not prevent TPS from suppressing LTP [data not shown ($n = 3$)].

We also examined whether OA interacts with the type of depotentiation obtained in the presence of AMPA receptor modulator BDP-12, a compound shown to enhance the strength of the re-

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versal process significantly, thereby enabling TPS delivered 15 min post-TBS to produce near complete depotentiation (Staubli and Chun 1996). As shown in Figure 3C, OA blocked the depotentiation; that is, the magnitude of LTP measured 30 min post-TPS in the test input was virtually identical in size to LTP in control pathways, despite the presence of BDP-12 during TPS. In contrast and as illustrated in the inset of Figure 3C, the degree of reversal in our original BDP-12 experiments that did not involve OA was almost complete ($>90\%$).

Extracellular application of adenosine, if administered within 1–3 min after TBS, prevents the formation of LTP (Arai et al. 1990a). Moreover, transient hypoxia occurring within 1–3 min after TBS, but not later, interferes with the stable expression of LTP, an effect that is blocked by antagonists of A_1 adenosine receptors (Arai et al. 1990b). These results support the notion that the vulnerability of LTP to reversal lasts only for a short time after induction and, moreover, suggest that TPS causes adenosine release or that its reversing effects require interactions with concentrations of the nucleoside normally present. This idea was tested here. In the presence of CPX, a selective A_1 adenosine receptor antagonist, TPS given 30 sec post-TBS to the test pathway was unable to reverse LTP that was $174.1 \pm 13.1\%$ of baseline 45 min after reversal attempts (Fig. 4A). CPX facilitated baseline synaptic responses but did not greatly enhance the magnitude of LTP, consistent with previous reports (Arai et al. 1990a; Larson et al. 1993). The effects of CPX on baseline responses seemed irreversible because prolonged

washout caused no decrement in EPSP size and repeated perfusions with CPX did not produce additional response enhancements. When the above protocol was repeated in slices that had already been perfused with CPX earlier, TPS was again unable to reverse LTP (Fig. 4B).

In a final step, the NMDA-receptor independence of LTP reversal by TPS was reassessed. We had found previously that AP5 does not interfere with the ability of TPS (60 sec) to cause depotentiation (Staubli and Chun 1996). However, this earlier experiment had been conducted with AMPA receptor modulator BDP-12 in the medium, and it is possible that in the presence of the drug, TPS produces a qualitatively different form of reversal. Moreover, work by others indicates that AP5 completely blocks depotentiation produced by TPS for 3 min (O'Dell and Kandel 1994) and by LFS (Wexler and Stanton 1993). Therefore, both these stimulation patterns were tested here. As illustrated in Figure 5A', LFS (900 pulses at 1 Hz) delivered to the test input 15 min after LTP induction produced significant depotentiation ($39.2 \pm 10.8\%$), an effect that was completely blocked when AP5 was present during reversal attempts (Fig. 5A''). In a next set of experiments, AP5 was tested in combination with a 60-sec TPS train, and as shown in Figure 5B', B'', AP5 clearly failed to block depotentiation. Moreover, as depicted in the inset of Figure 5B', the percent reversal obtained in the presence of AP5 was slightly larger ($36.6 \pm 11.2\%$; $n = 6$) than that observed with the same TBS–TPS interval in our original experiments conducted without AP5 ($27 \pm 5\%$, $n = 5$; see

Figure 4: Antagonism of adenosine A_1 receptors completely blocks LTP reversal by TPS. (A) Group data ($n = 5$) showing that in the presence of CPX, an adenosine A_1 receptor antagonist, TPS was unable to reverse LTP induced 30 sec earlier in the test input (S1). Note that CPX facilitated baseline synaptic responses of the control input (S2) by 25–35%, in an irreversible manner. (Insets) Superimposed EPSPs taken at the times indicated in the graph (calibration: 0.5 mV/5 msec). (B) Replication of the same experiment as shown in A, using a second application of CPX and a different slice from the same well ($n = 4$). Test responses were $156.3 \pm 4.7\%$ of baseline 45 min following TPS, compared with $99.7 \pm 4.3\%$ measured in the control input.

Figure 5: AP5 blocks reversal of LTP by LFS but not by TPS. (A') Fifty minutes after delivering a 15-min train of LFS to the potentiated test input, LTP was at $136.4 \pm 6.4\%$ of baseline compared with $161.0 \pm 4.8\%$ in the control input [paired $t(4) = 3.11$, $P < 0.02$]. (A'') The NMDA receptor antagonist AP5 completely blocked depotentiation produced by LFS. (B') LTP was induced simultaneously in two independent inputs, followed by delivery of a 1-min train of TPS in the presence of AP5 to the test input 10 min later, a delay at which depotentiation obtained under control conditions is still large enough that potential suppressive effects of AP5 can easily be detected (see Staubli and Chun 1996). AP5 was administered 5 min before TBS, because past experience indicates that complete NMDA receptor blockade in our interface-type recording chamber requires 15 min of incubation. LTP in the test input was $131.5 \pm 3.3\%$ of baseline 50 min after TPS compared with $154.2 \pm 8.2\%$ in the control input [paired $t(5) = 2.0$, $P < 0.05$]. (Inset) Percent reversal obtained with a 10-min TBS–TPS interval in the presence of AP5 was slightly larger than without AP5 [unpaired $t(9) = 0.73$, not significant]. Waveforms above graph illustrate superimposed EPSPs taken at the times indicated (calibration: 1.5 mV/5 msec). (B'') Same as in B', except that TPS was administered for 3 min. Again, AP5 did not interfere with depotentiation: LTP in the test pathway was $156.7 \pm 7.7\%$ of baseline compared with $124.5 \pm 3.6\%$ in control LTP 45 min after reversal attempts [paired $t(5) = 5.01$, $P < 0.002$].

Staubli and Chun 1996). Identical results were obtained in six additional experiments in which the duration of TPS was prolonged to 3 min (Fig. 5B''). If anything, AP5 facilitated rather than interfered with depotentiation; that is, the percent reversal obtained was $53.5 \pm 8.2\%$ ($n = 6$), slightly but not significantly larger than that produced by a 1-min train of TPS in the presence of AP5.

Discussion

The above results confirm earlier findings that short episodes of single-pulse stimulation pat-

terned at the frequency of the endogenous theta rhythm (TPS episodes) are capable of removing LTP in subpopulations of recently potentiated synapses. As shown previously, TPS had no lasting effect when applied to naive synapses and, equally important, did not cause heterosynaptic depotentiation. A new finding that emerged was that TPS of a minimal duration (15 sec) was required to produce measurable and stable depotentiation, whereas longer episodes increased the degree of synaptic depression by an additional amount until a fixed desaturation level was reached with 1 min of TPS, the magnitude of which depended on the temporal coupling between TBS and TPS [we had

shown previously that prolonging TPS from 1 to 3 min did not result in greater depotentiation (Staubli and Chun 1996)]. A fractional degree of induction and saturation are properties reminiscent of LTP produced by TBS, where each burst incrementally brings the synapse to a fixed ceiling level (Larson and Lynch 1986). However, an important difference between LTP induced by TBS and LTP reversal produced by TPS is that the latter does not require NMDA receptor activation. On the contrary, in presence of AP5 the degree of reversal was consistently larger compared with control depotentiation, an effect already observed in our previous study (Staubli and Chun 1996) but that did not reach statistical significance. In sum, it appears that theta bursts and theta pulses, two very similar stimulation modes that mimic activity patterns observed in behaving animals (e.g., Hill 1978; Otto et al. 1991), initiate very different cellular mechanisms and thereby have very different and opposing effects on potentiation, that is, either induce LTP or reverse it.

As an alternative method for reversing LTP, we used LFS consisting of 900 stimuli at 1 Hz delivered at 15 min, which caused depotentiation comparable in amount to that obtained, had TPS been applied at the same time point. However, LFS-induced depotentiation was completely blocked by AP5, in agreement with findings by others (e.g., Wexler and Stanton 1993). Long episodes of low-frequency stimulation are known to cause long-lasting depression of basal synaptic transmission, especially in immature tissue (for review, see Bear and Malenka 1994) but also in hippocampal slices from adult animals (e.g., Staubli and Ji 1996), and it is thus possible that they initiate cellular mechanisms different from those triggered by reversal paradigms such as TPS, which have no lasting effects on baseline responses. This hypothesis is consistent with data showing that AP5 (1) did not significantly interfere with depotentiation produced by 900 stimuli at 2 Hz, a pattern that by itself left basal transmission unaffected (Bashir and Collingridge 1994), and (2) suppressed depotentiation produced by 1000 stimuli at 1 Hz, a pattern that also caused a measurable and persistent (>45 min) depression of nonpotentiated responses (Fujii et al. 1991). On the other hand, the above idea is somewhat at odds with results obtained in the guinea pig where AP5 was found to block depotentiation triggered by 900 pulses at 5 Hz, a pattern that by itself did not produce a de-

pression but, rather, a small, long-lasting enhancement of basal synaptic transmission (O'Dell and Kandel 1994).

The observations that (1) prior TPS suppressed the formation of subsequent LTP and (2) this effect as well as LTP reversal by TPS were both blocked by phosphatase inhibitors are in agreement with those of O'Dell and Kandel (1994). In addition, our experiments established that the strength of the proactive inhibitory influence exerted by TPS diminished steadily and was lost within 5 min, a time course that closely paralleled the recovery of baseline responses from temporary depression produced by TPS. That prior synaptic activity may inhibit subsequent LTP and/or increase the threshold amount of synaptic activation required to elicit LTP has also been shown by others (Fujii et al. 1991; Huang et al. 1992). These latter studies, however, used patterns of afferent stimulation different from TPS, including some that cause long-lasting basal depression, and found that the suppressive effect on the induction of subsequent LTP took 1 hr to decay, in addition to being blocked by AP5. It is likely that different modes of synaptic activity can interfere with subsequent LTP induction via different routes by triggering qualitatively distinct processes.

A popular idea is that LTP, LTD, and depotentiation are processes that are at least partially controlled by the balance between the activity of critical kinases and phosphatases in the postsynaptic cell (e.g., Lisman 1989, 1994; Mulkey et al. 1993). In this view, protein kinases become activated following high-frequency stimulation used to induce LTP, whereas LTD is attributable to preferential activation of protein phosphatases triggered by low-frequency stimulation. Presumably then, TPS delivered after LTP induction elicits depotentiation by activating protein phosphatases that oppose and override the action of the kinase cascade triggered by high-frequency stimulation. In our experiments, OA had little effect on baseline responses or the induction or expression of LTP but did interfere with the proactive and retrograde effects of TPS. This suggests that short TPS episodes produce a low level of rapidly dissipating phosphatase activity that is capable of counteracting kinase activation triggered by a subsequent tetanus given during this critical time window. Similarly, TPS delivered *after* the tetanus has a lasting impact on synaptic strength only if the activated phosphatases are present during the same time window in which kinase action is required to es-

ablish LTP, which, according to our results, would be $\sim 15\text{--}30$ min.

Phosphatase inhibition in our experiment was only partially effective at preventing TPS from interfering with subsequent LTP induction or depotentiating recently tetanized synapses, probably because the incubation time with OA was insufficient. Slightly longer applications of OA were successful in fully counteracting the substantial depotentiation obtained with TPS delivered 15 min after LTP induction in the presence of AMPA receptor modulator BDP-12. A still unanswered question pertains to the mechanism whereby AMPA receptor modulators facilitate the reversal process. Considering the present findings, a link with protein phosphatases seems likely. An alternative is that the modulator alters the configuration of the AMPA receptor and thereby renders it more accessible to phosphatases triggered by TPS. If the receptor is the locus of LTP expression (for recent discussions, see Staubli et al. 1992; Ambros-Ingerson and Lynch 1993), and given that phosphorylation has been shown to modify the function of the non-NMDA-type glutamate receptor channels by increasing opening frequency and mean open time (Greengard et al. 1991), then the kinase/phosphatase balance might well be responsible for an initial phase of potentiation. From this it would follow that allosteric modulators that affect exposure of phosphorylation sites would have significant impact on potentiation, depotentiation, or both.

The blockade of the adenosine A_1 receptor was very effective at preventing depotentiation by TPS delivered immediately after induction of LTP, in agreement with earlier findings (Larson et al. 1993). This result combined with the observation that exogenous adenosine acting on A_1 receptors reverses LTP if applied shortly after LTP induction (Arai et al. 1990a), suggests that adenosine is normally released during TPS and has inhibitory effects on LTP stabilization. It is indeed an intriguing idea that high concentrations of adenosine receptors found at synapses in many telencephalic areas including hippocampus (Dunwiddie 1985; Deckert and Jorgensen 1988) regulate the processes whereby lasting modifications are imposed upon or erased from those synapses. Whether the influence of the A_1 receptor is mediated through protein phosphatases or instead involves a separate pathway acting on a target shared by the phosphatases is an open question. In any event, the pharmacological experiments have provided tools

and results with which to begin developing hypotheses about the chemistries underlying the LTP reversal effect.

Further developments of the type just discussed may also allow for tests of an issue that has been left unexamined in the 15 years since the discovery of LTP reversal (Barrionuevo et al. 1980), namely, what types of contributions to behavior might be made by an active process that erases synaptic changes? The observation that a naturalistic pattern of stimulation is quite effective in causing depotentiation encourages the suggestion that the phenomenon occurs in vivo. However, unlike the case for LTP itself, the reversal effect has no obvious (potential) correspondences in learning and memory. Thus, were drugs to be found that block the effect with some selectivity, it would be all the more interesting to test if and how they influence behavior.

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