Differing Mechanisms of Expression for Short- and Long-Term Potentiation

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Schulz, Paul E. and Jill C. Fitzgibbons. Differing mechanisms of expression for short- and long-term potentiation. J. Neurophysiol. 78: 321–334, 1997. Long-term potentiation (LTP) is a use-dependent form of synaptic plasticity that is of great interest as a cellular mechanism that may contribute to memory storage. It is the sustained phase of population excitatory postsynaptic potential induced by high-frequency stimulation (HFS). HFS can also induce short-term potentiation (STP), a decremental potentiation lasting ~15 min. It has been unclear whether STP is simply a reversible form of LTP elicited by subthreshold stimuli or whether it is an independently expressed form of synaptic plasticity. We have attempted to clarify the relationship between LTP and STP in the extracellular recording technique in area CA1 of the adult rat hippocampal slice preparation to test four predictions of the hypothesis that LTP and STP are expressed via the same mechanism. First, occluding LTP expression should block STP expression. Saturating LTP under six different conditions, however, did not occlude STP expression. Second, occluding STP expression should occlude LTP expression. The partial or full occlusion of STP by two maneuvers (increasing the stimulus intensity used for HFS or applying 3-isobutyl-1-methylxanthine), however, did not occlude LTP expression. Third, LTP increases and decreases paired-pulse facilitation (PPF), and STP should have the same effect. STP did not change PPF; however. The first three results, then, suggest that STP and LTP are expressed via different mechanisms. Fourth, STP should be maximal near the LTP induction threshold, and then decrease above it. Surprisingly, STP was maximal at or very close to the LTP induction threshold, but it did not decrease above this threshold. This relationship suggests the possibility that STP and LTP share an induction step(s). What is the function of the independently expressed STP? We find that LTP can be induced by two HFSs, each of which is subthreshold for LTP, if the second is given during STP from the first. This suggests that STP can temporarily lower the LTP induction threshold. Three lines of evidence, then, suggest that STP and LTP may be expressed via different mechanisms; however, the proximity of STP saturation to LTP induction suggests that they may share an induction step(s). STP may also have the very important function of temporarily lowering the LTP induction threshold. Finally, these data suggestion caution in interpreting LTP data obtained ~20–30 min after HFS, because they may be contaminated by STP, which appears to have different underlying mechanisms.

INTRODUCTION

Long-term potentiation (LTP) is a use-dependent form of synaptic plasticity that is of great interest as a cellular mechanism that may contribute to memory formation (Brown et al. 1988; Madison et al. 1991; Teyler and DiScenna 1984). LTP of Schaffer collateral fibers in hippocampal area CA1 is defined as the sustained potentiation, with a duration of >15 min, that can be induced by a short burst of high-frequency stimulation (HFS). The induction of LTP by 100 Hz HFS is blocked by the N-methyl-D-aspartate (NMDA) receptor antagonist aminophosphono-valerate (APV).

HFS can also induce two decremental phases of potentiation that are distinguished on the basis of time course and sensitivity to APV. The first is an APV-insensitive component termed posttetanic potentiation (PTP), which has a very short duration (~0.5–4 min) and is thought to be mediated by a simple presynaptic mechanism (Swandulla et al. 1991). The second decremental phase is blocked by APV, has a duration of ~10–15 min, and is referred to as short-term potentiation (STP) (Anwyl et al. 1989; Kauer et al. 1988; Malenka 1991).

Although LTP and STP can be separated on the basis of time course (sustained vs. decremental), it has not been clear whether STP is simply a reversible form of LTP elicited by subthreshold stimuli or whether it is an independently expressed form of potentiation. It would be useful to know whether LTP and STP are independently expressed. If they are, it would suggest caution in interpreting LTP data collected soon after HFS, which could be contaminated by STP and therefore produce misleading results. It would also raise the possibility that they perform different functions in memory storage.

In a number of experiments, researchers have examined the relationship between STP and LTP, and the majority of results have been interpreted as suggesting that STP and LTP are the same or similar forms of potentiation. The induction of both is APV sensitive (Anwyl et al. 1989; Malenka 1991) and requires an increase in postsynaptic intracellular calcium (Malenka et al. 1988). STP reversibly occludes LTP, and vice versa (Asztely et al. 1991; Collingridge et al. 1991; Gustafsson et al. 1989; Huang et al. 1992; Kauer et al. 1988). Both are associated with increases in the amplitude of miniature synaptic currents (Manabe et al. 1992). And the degree to which STP decays versus the remaining stable LTP varies under different conditions, which suggests that STP can be converted to LTP to different extents (Gustafsson and Wigström 1990; Gustafsson et al. 1989; Malenka and Nicoll 1990).

The results of other experiments, however, suggest that STP and LTP could be different forms of potentiation. Their induction thresholds differ (Malenka 1991). NMDA-induced STP is inhibited in high-calcium saline, whereas LTP is not (McGuinness et al. 1991a). An STP-like phenomena is induced by NMDA application alone (Kauer et al. 1988), but it has been difficult, although not impossible (Collingridge et al. 1991), to induce LTP through NMDA application. And second-messenger involvement may differ: protein...
kinase C activation enhances LTP (Loving and Routenberg 1988), but inhibits STP (Asztely et al. 1990; McGuinness et al. 1991b), and multiple kinase inhibitors block LTP (Malenka et al. 1989; Malinow et al. 1988, 1989), leaving an STP-like phenomenon (Malenka et al. 1989; Malinow et al. 1989; O’Dell et al. 1991; Schuman and Madison 1991). The kinase blocking experiments appear to argue strongly for differing induction steps for STP and LTP. It is not clear, however, whether the decremental potentiation induced in the presence of kinase inhibitors is the same as STP. At a minimum, STP and kinase-inhibitor-induced decremental potentiation differ markedly in duration (10–20 min vs. 30–60 min). In addition, even if they are identical, the kinase results could still be consistent with similar mechanisms of expression. LTP expression, for example, may require a kinase for stabilization. In the absence of an activated kinase, then, decremental potentiation (STP), mediated by the same mechanism, would result.

Because experiments examining the relationship between STP and LTP have produced conflicting results, and many results have multiple interpretations, the relationship between STP and LTP has remained unclear. Thus we have reexamined this relationship by testing four predictions of the hypothesis that STP is simply a reversible form of LTP induced by subthreshold stimuli. Three results suggest that STP and LTP are actually expressed via independent mechanisms. The fourth result, however, suggests that STP and LTP may have overlapping mechanisms of induction. Finally, we report that an important function of STP may be to temporarily lower the LTP induction threshold. Some early results have been presented in abstract form (Schulz and Johnston 1991, 1992).

METHODS

Preparation of hippocampal slices

Brains of adult Sprague-Dawley rats (50–150 g) were quickly removed and placed in iced saline. The hippocampi were dissected out and 400 μm-thick slices were made perpendicular to the septotemporal axis with a Vibratome (Technical Products International). Slices were transferred to a Haas-type (Haas et al. 1979) interface recording chamber (Medical Systems) at 32.5 ± 0.1°C (mean ± SE) and maintained with the use of standard procedures (Schulz 1997; Schulz et al. 1994, 1995).

Four bath salines were used. The normal-calcium solution contained (in mM) 120 NaCl, 3 KCl, 23 NaHCO3, 11 dextrose, 1.5 CaCl2, and 1.2 MgCl2. The three other salines contained similar base constituents (in mM): 120 NaCl, 2.4 KCl, 25 NaHCO3, and 10 dextrose. They differed, however, in CaCl2 and MgCl2 concentrations, which were 3 and 3 mM in the moderate-calcium saline, 4.5 and 1.5 mM in the high-calcium saline, and 1.5 and 4.5 mM in the high-magnesium saline. Picrotoxin (10 μM) was added to the last three salines to decrease γ-aminobutyric acid-A-mediated inhibition. The salines were gassed with 95% O2-5% CO2. The bathing solution sometimes contained 100 μM D-APV (Research Biochemicals, Natick, MA) or 10 μM 3-isobutyl-1-methylxanthine (IBMX), as indicated.

Extracellular recordings

Microelectrodes were pulled from 1.5-mm-OD glass tubing with the use of a Flaming/Brown micropipette puller (Sutter Instrument) and were filled with 750 mM NaCl (resistance 1–5 MΩ).

Stimulation was given at 20-s intervals via bipolar, Teflon-coated platinum stimulating electrodes (WPI stimulus generator and isolator, stimulus duration 50 μs). Extracellular population excitatory postsynaptic potential (pEPSP) recordings were made from stratum radiatum in area CA1. The data were filtered at 5 kHz and recorded on-line with either a DEC-11/23 or a NeXt computer with software written in Basic.

Stable pEPSPs were obtained with a stimulus intensity that initially yielded either a 1.3- or 2.6-nV pEPSP. A single stimulus intensity was used throughout each experiment (with the few exceptions noted), including for HFS, in an effort to stimulate the same axons throughout the experiment. Using the single intensity allowed STP to be compared across HFS. For Figs. 1–4 and 6, HFS consists of 10 trains (100 Hz, 50 ms) delivered at 200-ms intervals for a total of 50 stimulations over 2 s. For the later experiments, HFS was given as a single, continuous stimulation at 100 Hz for the durations indicated (0.025–2.0 s). To saturate LTP, HFS was given at 20- to 40-min intervals until no additional LTP was elicited. In some experiments, LTP was saturated more rapidly by administering six HFSs over 6 min followed by HFS every 20 min.

Data were obtained at 20-s intervals throughout the experiments, but were analyzed in 1-min bins for clarity in graphing. For some experiments, the data before HFS, and >15 min after HFS, were put in 5-min bins. In addition, the first point after HFS, which was the only one containing PTP, was analyzed as a single trace to more easily distinguish PTP from STP.

Paired-pulse facilitation (PPF) was measured with the use of a 55-ms interstimulus interval. To examine PPF during STP in the absence of LTP, LTP was saturated before the measurements.

Data analysis

To obtain suitable pEPSPs, an attempt was made to clearly identify the fiber volley as being separate from the initial pEPSP. Early results have been presented in abstract form (Schulz 1997; Schulz et al. 1994, 1995). Because experiments examining the relationship between STP and LTP have produced conflicting results, and many results have multiple interpretations, the relationship between STP and LTP has remained unclear. Thus we have reexamined this relationship by testing four predictions of the hypothesis that STP is simply a reversible form of LTP induced by subthreshold stimuli. Three results suggest that STP and LTP are actually expressed via independent mechanisms. The fourth result, however, suggests that STP and LTP may have overlapping mechanisms of induction. Finally, we report that an important function of STP may be to temporarily lower the LTP induction threshold. Some early results have been presented in abstract form (Schulz and Johnston 1991, 1992).

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DIFFERING MECHANISMS OF EXPRESSION FOR STP AND LTP

FIG. 1. Occlusion of long-term potentiation (LTP) does not occlude the expression of short-term potentiation (STP). A: high-frequency stimulation (HFS) was administered repeatedly at a single stimulus intensity that eventually resulted in LTP saturation (Satn: slope increase = 204%; n = 4, high-calcium solution, initial population excitatory postsynaptic potential (pEPSP) = 1.3 mV, mean ± SE). Inset: raw voltage traces from a typical experiment (each is an average of several traces). B: pEPSP slopes of the 1st 7 HFSs in A were normalized (pre-HFS slope = 100%), the time of HFS was set equal to 0, and slopes are superimposed. C: to compare STP across HFS, LTP (slope 15 min after HFS) was subtracted from each curve in B and is plotted in C (see text for example). C demonstrates that normalized STP is of constant magnitude and duration from baseline through LTP saturation, despite occlusion of LTP, which suggests differing mechanisms of expression for STP and LTP.

repeatedly at 20-min intervals until LTP was saturated (Fig. 1A, initial pEPSP = 1.3 mV, high-calcium saline, LTP = 304%, baseline = 100%, n = 4). Despite the occlusion of LTP, STP expression persisted. In fact, the absolute STP magnitude increased across sequential episodes of HFS; for example, the STP associated with the seventh HFS is 0.5 mV/ms (2.8 - 2.3 = 0.5) versus 0.3 mV/ms for the first HFS (1.4 - 1.1 = 0.3).

To compare STP across the seven HFSs that start from differing baselines due to LTP, STP was normalized to the pEPSPs obtained just before HFS. The time of HFS was set equal to zero, and the resulting curves are shown superimposed in Fig. 1B. To examine STP in isolation from LTP, which is defined as the average size of the pEPSP 15–20 min after HFS, LTP was subtracted from each curve; the results are plotted in Fig. 1C. As an example, the average pEPSP 15–20 min after the first HFS was 141% of the baseline pEPSP. Thus 141% was subtracted from each point in the HFS 1 curve in Fig. 1B to produce the HFS 1 curve in Fig. 1C. Surprisingly, the results indicate that normalized STP is constant from the first HFS through LTP saturation. Thus LTP saturation does not appear to effect STP.
FIG. 2. LTP occlusion under other conditions also does not occlude STP expression. HFS was administered repeatedly with a single stimulus intensity that initially yielded pEPSP amplitudes of 1.3 mV (A, n = 7 and C, n = 12) or 2.6 mV (B, n = 8 and D, n = 7), either in normal-calcium (A and B) or moderate-calcium (C and D) saline. Data are displayed as in Fig. 1C. All axes have same scale for convenience of comparison. Under each condition, the STP elicited by all HFS was of similar magnitude and duration. Less STP was expressed, however, with HFS at 2.6 mV (B and D) vs. 1.3 mV (A, C, and Fig. 1) . Even when less STP was expressed, though, LTP magnitude was identical (compare distances of baseline points below ± ± ± , which is equivalent to LTP magnitude).

The same results were obtained when the effect of LTP saturation on STP was examined under other conditions. It was examined in the normal-calcium (Fig. 2, A and B, n = 7 and 8, results presented as in Fig. 1C), the moderate-calcium (Fig. 2, C and D, n = 12 and 7), and the high-magnesium saline (data not shown, n = 4). It was also examined with the use of two different stimulus intensities to follow and administer HFS: one that induced a 1.3-mV pEPSP (Figs. 1 and 2, A and C) and one that induced a 2.6-mV pEPSP (Fig. 2, B and D, high-magnesium data not shown). STP appears to be the same across HFS under each condition, although this conclusion is less convincing under the conditions in which less STP was elicited (Fig. 2, B and D).

The finding that STP remains constant across HFS is demonstrated by plotting LTP versus STP, across HFS, for all six conditions tested (Fig. 3). Using HFS with a 1.3-mV pEPSP in high-calcium as an example (open squares), the first HFS induced 41% LTP and 26% STP. Subsequent HFS induced less LTP until it was saturated (saturation = 0 on x-axis). Nonetheless, the STP elicited remained relatively constant, which is reflected in the horizontality of the regression line. The horizontality of all six regression lines indicates that saturating LTP has little or no effect on STP expression. The hypothesis that LTP and STP are expressed via the same mechanisms, in contrast, predicts that LTP induction would decrease STP so that the regression lines would terminate at the origin. The finding that STP is not altered by occluding LTP less convincing under the conditions in which less STP was elicited (Fig. 2, B and D).
**DIFFERING MECHANISMS OF EXPRESSION FOR STP AND LTP**

**FIG. 3.** STP magnitude is independent of LTP magnitude. HFS was given repeatedly with a 1.3-mV (○, □, △) or a 2.6-mV (●, †, •) pEPSP in 4 different solutions. Resulting STP and LTP amplitudes were averaged across experiments and are plotted. LTP was defined as potentiation present 15–20 min after HFS. STP was defined as an average of the 3 highest points after HFS, excluding those containing posttetanic potentiation (PTP). Points at right on X-axis are initial HFSs under each condition, which produce the most LTP. Points at left are subsequent HFSs that produce less LTP as saturation is approached (saturation = 0 on the X-axis). Horizontality of regression lines indicates that STP and LTP magnitude are independent.

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been maximized, or the decremental potentiation under study might have been PTP instead of STP. We examined each possibility as follows.

**MAXIMIZING STP EXPRESSION.** One explanation for not detecting a decrease in STP as LTP was saturated is that maximal STP may not have been elicited under the conditions of study. For a number of reasons, it may then be difficult to detect a decrease in STP. Thus we sought conditions under which greater STP might be elicited. Two maneuvers hypothesized to enhance STP were increasing bath calcium and increasing the stimulus intensity used for HFS. Both should enhance postsynaptic calcium influx during HFS, which has been reported to increase STP (Malenka 1991).

The effect of increasing bath calcium was examined by comparing the STP elicited in the normal-calcium (Fig. 2A), the moderate-calcium (Fig. 2C), and the high-calcium (Fig. 1) salines. The STP evoked by the first HFS under each condition is shown in Fig. 4A (initial pEPSP = 1.3 mV). Despite increasing bath calcium threefold (from 1.5 to 4.5 mM), STP was only marginally greater in the high- versus normal-calcium saline, which may simply have been due to greater PTP.

The effect of increasing the stimulus intensity used for HFS was examined by comparing the results of HFS with a 1.3-mV pEPSP (Figs. 1 and 2, A and C) versus a 2.6-mV pEPSP (Fig. 2, B and D, high-magnesium data not shown). The same stimulus intensity was used to follow the pEPSPs and administer HFS. Larger stimulus intensities presumably increase the number of active fibers and thus increase cooperativity and postsynaptic calcium influx. But less STP was elicited by HFS with the greater stimulus intensity.

We attempted to elicit additional STP in a third way. After LTP was saturated by our standard induction protocol, we tested whether additional STP and LTP could be elicited by giving another HFS with a greater intensity or a longer duration. This is illustrated in Fig. 4B, where STP alone was elicited after LTP was saturated (○). Then stimulus intensity was increased by an average of 70%, an additional HFS was administered, and stimulus intensity was returned to baseline for recording. No additional STP or LTP was elicited (●). Another HFS at the original stimulus intensity was given to verify that the slice was still capable of producing the original STP magnitude (△). The effect of increasing the duration of HFS was examined next. HFS was given for 1 s at 100 Hz, and was repeated three times for a total of 300 stimuli (vs. 50 stimuli over 2 s in the original protocol). Three conditions were tested: two slices from Fig. 1, six slices from Fig. 2C, and two slices from Fig. 2D (data not shown). No additional STP or LTP was elicited.

The lack of effect on STP of these four maneuvers was initially puzzling until it was recognized that researchers in the study on which our hypotheses were based (Malenka 1991) used sub-LTP-threshold stimuli, whereas we used supra-LTP-threshold stimuli. An explanation for our results, then, may be that when LTP is elicited, STP is already maximal. Increasing calcium influx further, then, would not produce additional STP. This important possibility is explored in greater detail later. We concluded here that maximal STP is apparently elicited by our LTP induction protocol.

**MAXIMIZING LTP EXPRESSION.** A second explanation for the lack of effect of LTP saturation on STP is that not enough LTP was elicited. Several measures were taken, however, to maximize LTP magnitude. First, LTP was studied under conditions thought to increase it, such as testing in the presence of picrotoxin and increased extracellular calcium. Second, the magnitude of LTP elicited at saturation was compared with that elicited by other groups, and they were found to be similar. And third, after LTP was saturated by our standard induction protocol, we tested whether additional LTP could be elicited by HFS with a greater intensity or a longer duration. As noted above, neither maneuver elicited additional LTP. We conclude that there are no obvious ways to elicit additional LTP.

**PTP VERSUS STP.** A third explanation for the lack of effect of LTP saturation on STP is that the decremental potentiation under study was actually PTP instead of STP. This possibility was addressed by examining the time course of PTP in the normal- and high-calcium salines. HFS was given in the presence of 100 μM D,L-APV, which blocks both STP and
LTP. In normal calcium, the duration of PTP was very brief, in agreement with previous observations (Malenka 1991), only being present during the first point measured 5–15 s after HFS (Fig. 4C, ●, n = 5). In high calcium, PTP has a duration of 30 s–2 min (data not shown, n = 5). Thus all of the decremental potentiation observed after the first minute or two is STP and not PTP.

In summary, multiple maneuvers failed to elicit additional STP or LTP, suggesting that STP and LTP are maximized in these experiments. PTP is also of short duration, so that it would not have interfered with the results. Thus it appears that the finding that LTP saturation does not occlude STP can be most easily explained by the two forms of potentiation having independent mechanisms of expression.

Occluding STP does not occlude LTP expression

A second prediction of the hypothesis that STP and LTP are expressed via the same mechanism is that STP occlusion will occlude LTP. It was already observed that STP is partially occluded by increasing the stimulus intensity used for HFS, and that this does not occlude LTP (Fig. 2, B and D). We sought another circumstance under which STP could be elicited despite completely occluding LTP.

IBMX was empirically found to occlude STP expression about one-fourth of the time (n = 18). An example is shown in Fig. 5A, where LTP was elicited with STP (1st †), IBMX was washed in, and an additional HFS was given (2nd †). It elicited LTP despite completely occluding STP. After IBMX washout (3rd †), STP returned to normal. The first two HFSs from Fig. 5A are normalized in Fig. 5B1. The normalized STP elicited after IBMX washout is shown in Fig. 5B2. The occlusion of STP by IBMX is specific, because when STP is produced by one HFS under control conditions, it is never occluded by a second HFS given at the same intensity and duration (n = 102). These IBMX results were recently confirmed in part (Blitzer et al. 1995).
Differential effects of STP and LTP on PPF

A third prediction of the hypothesis that STP and LTP are expressed via the same mechanism is that they should have the same effect on the expression of PPF. PPF is an increase in a second EPSP when it follows shortly after a first, which is thought to be presynaptically mediated. LTP alters PPF in a very predictable way: it increases or decreases PPF depending on the initial PPF value (Buonomano and Merzenich 1996; Kleschevnikov et al. 1997; Schulz 1997; Schulz et al. 1994, 1995). PPF averaged across all slices remains constant, however, as demonstrated by many groups (Arai et al. 1994; Ghijsen and Da Silva 1991; Manabe et al. 1993; McNaughton 1982; Muller and Lynch 1989; Schulz et al. 1995; Zalutsky and Nicoll 1990).

An example of a decrease in PPF associated with LTP is shown in Fig. 6. D and E (D–F are reproduced here for convenience from Schulz et al. 1995). The slopes of the first and second paired stimuli are shown over time in Fig. 6.D. PPF for that slice is shown over time in Fig. 6E, where the slope of Stim 1 increased from 0.66 to 1.08 with the first six HFSs, and the slope of Stim 2 increased from 0.86 to 1.34 so that PPF decreased from 30% to 24%. HFS to other slices produced an increase or a decrease in PPF with LTP (Fig. 6F). The pattern that emerged to explain the increases or decreases in PPF is that greater initial PPF is associated with decreases in PPF (Fig. 6F, right) and greater LTP. And lesser initial PPF is associated with increases in PPF (Fig. 6F, left) and less LTP.

The effect of STP on PPF was examined in isolation by first saturating LTP. The time course of the resulting STP is demonstrated in Fig. 6A (n = 8, high-calcium saline). PPF was unchanged during STP, except for the first point after HFS, which decreased due to PTP (Fig. 6B). Another report also found no change in PPF during STP (Colino et al. 1992). The lack of change in average PPF with STP (Fig. 6B) could be misleading if PPF both increased and decreased as it does with LTP (Fig. 6F). To address this possibility, the change in PPF associated with STP in each individual slice is plotted versus initial PPF in Fig. 6C (●). PPF values were obtained >2 min after HFS so that they are not contaminated by PTP. PPF was also examined during STP in the moderate-calcium saline; those data are plotted in Fig. 6C as open circles. A linear regression line for all points is drawn and follows the equation $y = -0.02x + 0.07$ ($n = 11$, $r^2 = 0.08$, $P > 0.20$). The relatively horizontal slope of $-0.02$ contrasts with that of Fig. 6F, where the slope is $-0.70$. STP and LTP have very different effects on PPF, then, suggesting for a third time that STP and LTP have differing mechanisms of expression.

**STP saturates at, or very near, the LTP induction threshold**

Tests of the first three predictions of the hypothesis that STP and LTP are similarly expressed suggested the opposite result, i.e., that they are, in fact, independently expressed. The fourth prediction has three parts: 1) STP has a lower threshold than LTP, 2) increases in the duration or intensity of HFS will increase STP up to the LTP induction threshold, and 3) HFS beyond that will be associated with decreases in STP. Those decreases will occur as more synapses develop the persistent changes of LTP, leaving fewer synapses to express STP. But if the hypothesis is wrong and the STP cascade is independent of LTP, then STP will continue to increase with each HFS beyond the LTP threshold. This is because the induction of LTP with one HFS will produce greater depolarization during the next HFS. The three parts of this prediction were tested with the use of two protocols.

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**FIG. 5.** Occluding STP expression does not occlude LTP expression. A: STP and LTP were elicited by the 1st HFS (†). Twenty minutes later, 3-isobutyl-1-methylxanthine (IBMX) was washed in and out without stimulating the slice. Ten minutes later, stimulation was restarted. After a suitable baseline, HFS was given again, and LTP was elicited without STP. HFS 20 and 40 min later elicited STP again. B1: 1st and 2nd HFSs from A are normalized and superimposed. They demonstrate a loss of STP despite preservation of LTP. B2: 3rd HFS from A is normalized and demonstrates that STP returned as IBMX washed out. That IBMX can block STP without occluding LTP suggests differing mechanisms of expression for STP and LTP. Note that IBMX partially or completely blocked STP only 7 of 18 times, so that no conclusion can be drawn regarding the mechanism underlying STP expression.

The second argument, then, for differing mechanisms of expression for STP and LTP is that the partial or complete occlusion of STP does not occlude LTP expression. Note that the mechanism by which IBMX sometimes occludes STP is unclear. Because the block is not consistent, it does not support a particular mechanism for STP expression at this time.
FIG. 6. Differential effects of STP and LTP on paired-pulse facilitation (PPF). A and B: STP was elicited in isolation from LTP (A) and had no effect on average PPF (B, n = 8). C: PPF for individual slices also did not change. Slices were tested in moderate calcium (open circles, n = 3) or high calcium (●, n = 8). Slope of linear regression line is −0.02 ($r^2 = 0.08, P > 0.20$). D: in contrast, LTP routinely elicits changes in PPF as shown in the example where both paired pulses are plotted. E: PPF for slice in D decreased as LTP was induced. F: changes in PPF with LTP occur in a regular way (n = 100). Slope of linear regression line is −0.70 ($P < 0.001$). Data in D–F are reproduced from Schulz et al. (1995). Differential effects of STP and LTP on PPF suggest differing mechanisms of expression.

In the first protocol, HFS was initially given with a low stimulus intensity that either produced no change in the pEPSP or induced a little STP without LTP. Successive HFSs were then given with progressively greater stimulus intensities. An example is shown in Fig. 7. HFS at the lowest intensities (Fig. 7A, 0.3 and 0.5 mV) did not appear to elicit STP. Higher stimulus intensities produced increasing amounts of STP (0.9, 1.1, and 1.3 mV). And finally, greater stimulus intensities elicited LTP (1.5, 1.7, and 1.9 mV) until it was saturated (2.1 mV). Normalizing the results of several HFSs from Fig. 7A and superimposing them (same method as Fig. 1C) demonstrates that less STP was induced below
the LTP threshold (Fig. 7B, 1.3 mV) versus above the LTP threshold (1.5 and 1.9 mV). In addition, the STP elicited above the threshold was of constant magnitude.

These results are consistent with the first two parts of the fourth prediction, i.e., the STP threshold is below that of LTP, and increases in stimulus intensity lead to increases in STP until LTP is elicited. But the third part was incorrect, i.e., STP did not decrease above the LTP threshold. Instead, it remained constant (1.5 and 1.9 mV). The interpretation of this experiment may be complicated, however, by having increased the stimulus intensity across HFS, which could recruit new neurotransmitter release sites. Thus the experiment was repeated, varying the duration of HFS rather than the intensity.

In the second protocol, then, the duration of HFS was progressively increased. Figure 8A demonstrates that HFS for successively longer durations (0.02–1.5 s) produced increases in STP, and then decreases. Normalizing, subtracting LTP, and superimposing this data for three of the HFSs (as in Fig. 1C) indicates that the greatest STP was obtained with HFS for 0.1 s, which was at the LTP induction threshold (Fig. 8B). Less STP was associated with earlier (0.025) and later (1.55) HFSs.

Another example of a decrease in STP with longer HFS is shown in Fig. 8C. This slice was given HFS from 0.1 to 0.5 s. The first HFS produced only STP. The second produced STP with LTP. Longer-duration HFS leads to additional LTP with less STP (0.4 and 0.5 s). The normalized pEPSP slopes for the 0.2- and 0.5 s-HFS are superimposed in Fig. 8D and demonstrate the decrease in STP with the longest HFS.

To demonstrate that STP, and not PTP, was elicited by the longest HFS, HFS was given for 0.5 s again to the slice in Fig. 8C in the presence of APV. The HFSs with and without APV are superimposed in Fig. 8E. PTP was only present during the first point after HFS, so that its contribution to decremental potentiation can be ignored.

Figure 8F is a summary plot of the STP elicited by successful HFS of increasing duration (n = 13). Because the LTP induction threshold differed between slices, thresholds were aligned by giving the threshold HFS a value of zero on the X-axis. The HFS before and after that point were given negative and positive numbers, respectively. STP magnitude (Y-axis) was derived by normalizing and subtracting LTP (as in B, D, and E) and then integrating the area under the first 20 min of the resulting curves. The means ± SE for all experiments are plotted. The figure demonstrates that there is an inverted U relationship between HFS and STP magnitude, which has a maximum very close to, if not exactly at, the LTP induction threshold.

At first, the decreases in STP as LTP is induced (Fig. 8) appear to conflict with Figs. 1–3, where it was shown that LTP saturation does not occlude STP. In Figs. 1–3, however, the stimulus intensity and duration were held constant across HFS, whereas in Fig. 8 the duration of HFS was increased across successive HFS. Nonetheless, because Fig. 8 suggests that STP can be occluded by LTP, we tested whether those decreases in STP are reversible. They would not be reversible if LTP occluded STP. HFS was given to the example slice in Fig. 9 for 0.2 s, which induced STP. Then HFS was given for a longer duration (1.0 s), which induced LTP and partially occluded STP. And finally, a shorter-duration HFS (0.4 s) again induced more STP. This indicates that the decrease in STP with longer-duration HFS is not due to a permanent occlusion of STP by LTP.

There was also concern that STP might actually have increased in Fig. 8F with the longer-duration HFS, but the increase might have been hidden. A large potassium efflux associated with HFS, for example, might have nonspecifically depressed synaptic activity for a period of time. To test this hypothesis, two inputs were monitored in a single slice as progressively longer-duration HFSs were given to one input. An example is shown in Fig. 10A. Increasing the duration of HFS eventually led to a decrease in the amount of STP expressed in one input (HFS for 1.50 s). Nonetheless, there was no decrease in the synaptic activity of the second, independent input as measured by the same recording electrode. Averaging three experiments performed as in Fig. 10A produced the plot in Fig. 10B, which also demonstrates that the decreases in STP in the first input are input specific and are not due to a generalized depression of synaptic activity.

We conclude from the experiments of Figs. 7–10 that STP saturates at, or very near, the LTP induction threshold. This result is consistent with the hypothesis that STP and LTP are expressed through the same mechanism. But that hypothesis also predicts that STP would then decrease with subsequent HFS, even with the use of a constant duration and
intensity, because more synapses would have the persistent changes of LTP. And that did not happen (Figs. 1–3). On the other hand, complete independence of STP and LTP could result in STP continuing to increase beyond the LTP induction threshold. This is because LTP from each HFS would increase synaptic activity during subsequent HFS, producing more STP. And that did not happen either (Figs. 7–10). One hypothesis that may reconcile these findings is that STP and LTP share an initial induction step(s), but then, as suggested by three lines of evidence, diverge to be expressed through independent mechanisms. Because they are independently expressed, STP would not decrease beyond the LTP induction threshold. And because they overlap in an early step(s), there is a relationship between when LTP is induced and when STP saturates.

**STP lowers the LTP induction threshold**

If STP is, in fact, an independently expressed form of potentiation, it raises the question of why two different NMDA-dependent forms of synaptic plasticity are necessary in the hippocampus and what their respective roles are. One hypothesis regarding the function of STP is that it might allow two subthreshold stimuli to induce LTP if they occur near each other in time. This was initially considered to be unlikely because of a report indicating that frequent HFS would increase synaptic activity during subsequent HFS, producing more STP. And that did not happen either (Figs. 7–10). One hypothesis that may reconcile these findings is that STP and LTP share an initial induction step(s), but then, as suggested by three lines of evidence, diverge to be expressed through independent mechanisms. Because they are independently expressed, STP would not decrease beyond the LTP induction threshold. And because they overlap in an early step(s), there is a relationship between when LTP is induced and when STP saturates.

**FIG. 8.** STP saturates at, or very close to, LTP induction threshold. A: HFS of successively longer duration produced an increase in STP and then a decrease. B: maximum STP was elicited at LTP induction threshold (0.10 s), and less STP was elicited by earlier (0.02 s) or later (1.50 s) HFS. C and D: another example of a decrease in STP with longer HFS (0.5 s). E: an additional HFS was given to the slice in C for 0.5 s with APV present. PTP elicited had a duration of only 1 min, indicating that the decremental potentiation in C is due to STP, not PTP. F: STP magnitude is plotted vs. HFS number for slices given HFS for successively longer durations (n = 13). LTP thresholds differed, so slices are aligned by assigning a value of 0 on the X-axis to threshold HFS. HFS before and after have negative and positive numbers, respectively. STP magnitude is area under potentiation curves derived by normalizing them and subtracting LTP. Maximum STP occurs near LTP induction threshold.
LTP by one or several HFSs does not have an obvious effect on STP. But concluding that LTP saturation has no effect on STP expression requires more rigorous testing. Accordingly, many measures were taken here to maximize both STP and LTP expression so that if LTP were to occlude STP expression, it would be detected. These measures included saturating LTP in a number of different salines, with the use of two different initial stimulus intensities, giving HFS with an increased stimulus intensity and duration after LTP was thought to be saturated, separately analyzing subsets of slices that had the most LTP, comparing the magnitudes of LTP at saturation to those previously reported, and testing for even small changes in STP by normalizing and superimposing the results of each HFS. Despite intensive study, however, we were unable to find any condition under which LTP saturation, by a constant-intensity and -duration HFS, led to an occlusion of STP expression. This suggests that STP and LTP are expressed via different mechanisms.

When HFS intensity (Figs. 2, 3, and 7) or duration (Figs. 8–10) were increased beyond the LTP induction threshold, however, STP expression decreased. This decrease is not due to a permanent occlusion of STP by LTP, however, because decreasing HFS duration returns STP to its baseline magnitude (Fig. 9). It is also not due to a nonspecific depression of synaptic activity because it is input specific (Fig. 10). The decrease in STP may have been an artifact, however, according to the following reasoning. Subtracting LTP from normalized potentiation assumes that the onset of LTP is abrupt. It may instead have

**D I S C U S S I O N**

There are three main findings of this study. First, three lines of evidence suggest that STP and LTP are expressed via different mechanisms: occluding LTP does not occlude STP expression, occluding STP does not occlude LTP expression, and STP and LTP have different effects on PPF. Second, this study demonstrates that STP saturation occurs either at, or very close to, the LTP induction threshold. This could hardly be coincidental, and suggests that STP and LTP may share an initial induction step(s). Finally, this study demonstrates that the pairing of two HFSs, which individually only induce STP, may result in the induction of LTP. This suggests that STP lowers the LTP threshold for a period of time, which may have very important consequences for synaptic efficacy.

**Differing mechanisms of expression**

It has been suggested that if LTP and STP are expressed via the same mechanism, then LTP saturation ought to occlude STP expression (Stevens 1993). Inspection of the figures in many studies suggests that the routine induction of

**FIG. 9.** Depression of STP by long-duration HFS is reversible. HFS for 0.2 s elicited STP. Another HFS for 1.0 s elicited LTP and partially occluded STP. A final HFS for 0.4 s elicited greater STP. This indicates that the depression of STP by the 1.0-s HFS was not due to permanent occlusion of STP by LTP, as would occur if STP and LTP were similarly expressed.

**FIG. 10.** Decrease in STP with longer-duration HFS is input specific. A: HFS of successively longer duration produced an increase in STP and then a decrease. Nonetheless, there was no change in a 2nd independent input, indicating that decreases are input specific. Slope of input 1 (●) and amplitude of input 2 (○) are plotted. B: average of 3 similar experiments is plotted.
FIG. 11. STP lowers LTP induction threshold. A and B: a single HFS only induced STP. Two HFSs of same intensity and duration, separated by 1 (A) or 3 (B) min, often resulted in LTP. C: superimposition of normalized group data for 1-min separations demonstrates LTP after paired HFS (●), but not single HFS (○).

An onset with a time constant of a couple of minutes, for example. Then as LTP increases across HFS, its relative contribution to potentiation may increase if the contribution by STP remains constant. STP, then, might appear to decrease. But regardless of why STP decreases under those two conditions, it does not appear to effect the conclusion that, when HFS is given with a constant intensity and duration (Figs. 1–3), STP is not occluded by LTP.

A second line of evidence suggesting that STP and LTP are expressed via independent mechanisms is that STP occlusion does not affect LTP expression. This was demonstrated in two sets of experiments. First, there was a partial occlusion of STP when the stimulus intensity (Figs. 2, 3, and 7) or duration (Figs. 8–10) used for HFS was increased, and it did not occlude LTP expression. And second, IBMX occasionally completely occluded STP, and this also did not block LTP expression (Fig. 5). Again, it should be noted that IBMX did not always block STP, so that no conclusion can be drawn from these experiments with regard to the mechanism underlying STP expression.

A third line of evidence suggesting differing mechanisms of expression for STP and LTP is that STP does not affect the expression of PPF (Fig. 6, A–C) the way that LTP does (Schulz 1997; Schulz et al. 1994, 1995). The lack of effect of STP on PPF (Colino et al. 1992), the positive effect of LTP on PPF in individual slices (Buono-

FIG. 12. Induction of LTP by paired HFS, which individually only induced STP, is not due to a longer total duration of HFS. A: HFS for 0.1, 0.3, and 0.5 s did not elicit LTP. Two HFSs for 0.1 s delivered 1 min apart, however, did. This occurred despite total HFS duration (0.2 s) being less than for the 0.3- or 0.5-s HFS. B: experiment in A was performed in reverse order. Two HFS for 0.05 s induced LTP, whereas another HFS for the same total duration (0.1 s) did not. Two more HFS for 0.05 s induced a small additional amount of LTP.
STP and LTP are differentially expressed. This conclusion has two important implications. First, to understand the mechanisms underlying LTP, it is important to separate LTP into components that can be studied individually. This study divides NMDA-dependent, HFS-induced potentiation into two processes: decremental STP and sustained LTP. Other studies have suggested that LTP may, in turn, be divided into an early and a late component. Second, these data may add to our understanding of the conflicting data in the literature regarding the current intensely debated question of the loci of LTP expression. If STP and LTP have different mechanisms and loci of expression, then data regarding “LTP” could be tainted by STP if those data are obtained too soon after HFS. The maximum duration of STP is not clear, but we often observe a duration of 20–30 min. In Fig. 8C, for example, HFS for 0.2 s elicited 30 min of STP.

**STP and LTP may share induction steps**

The fourth prediction of the hypothesis that STP and LTP are similarly expressed had three parts. First, the threshold for STP induction will be lower than for LTP. Second, as HFS intensity or duration is increased, STP magnitude will increase up to the LTP induction threshold. Once the threshold is met, then, rather than increasing the reversible STP, LTP will be expressed. And third, further HFSs above the LTP threshold will decrease STP because they will produce more LTP, thereby leaving fewer release sites available to express STP.

Part one of this fourth prediction was confirmed. The induction threshold for STP is markedly lower than for LTP (Figs. 7–12) (Malenka 1991), and both are APV sensitive (Fig. 4C) (Anwyl et al. 1989; Reymann and Matthies 1989; Reymann et al. 1989). But both findings could be explained by similar or dissimilar mechanisms of induction. Tests of part three yielded results that were opposite to the fourth prediction, i.e., STP did not decrease as LTP was induced, at least when HFS intensity and duration were held constant (Figs. 1–3). This argues for differing mechanisms of expression for STP and LTP.

Part two was then examined in detail. Careful testing of the magnitude of STP versus the LTP induction threshold showed that, in fact, STP is maximal at, or very near to, the LTP induction threshold (Fig. 8E). This result suggests a relationship between STP and LTP. But if they were the same form of plasticity, then STP would decrease with subsequent HFS because more release sites would have the persistent changes of LTP. These release sites would therefore be unavailable for STP. But that did not happen (Figs. 1–3). One hypothesis to explain these results is that STP and LTP have an overlapping mechanism(s) of induction. Then, as suggested by three other lines of evidence, STP and LTP diverge to be expressed through independent mechanisms. Thus, their overlapping induction step(s) could produce a close relationship between STP saturation and LTP induction. But HFS above the LTP threshold would not occlude STP expression, because it is independent of LTP.

**STP lowers the LTP induction threshold**

Because STP appears to be an independently expressed form of synaptic plasticity, it must have a specific function. We investigated the possibility that its acts to raise or lower the LTP induction threshold. It has already been shown that frequent, repeated (5 times) induction of STP can raise the LTP threshold (Huang et al. 1992). Nonetheless, we investigated whether administering only a single STP-inducing HFS might lower the LTP threshold for a short period of time. Our data indicate that, in fact, the STP induced by a single HFS lowers the LTP induction threshold for at least a few minutes so that a second, previously subthreshold HFS can produce LTP (Fig. 11). STP, then, may act as a coincidence detector. Such a role for STP has important implications for our understanding of synaptic plasticity and needs to be considered in the neural modeling of changes in synaptic weights.

In conclusion, these experiments raise the interesting possibility that STP and LTP share an initial step(s) in their induction cascades, and then diverge to be expressed via different mechanisms. Their differing mechanisms of expression suggest that data regarding LTP could be misleading if it is obtained while STP is still present. The independent mechanisms of expression of STP and LTP also suggest that they could serve different functions. At least one important function of STP appears to be that it can temporarily lower the LTP induction threshold. By modifying that threshold in an ongoing way, STP could have a direct, significant impact on synaptic efficacy.

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