Interactions between paired-pulse facilitation, low-frequency stimulation, and behavioral stress in the pathway from hippocampal area CA1 to the subiculum: Dissociation of baseline synaptic transmission from paired-pulse facilitation and depression of the same pathway

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The interaction between low-frequency stimulation (LFS; 900 stimuli at 10 Hz) of field excitatory postsynaptic potentials (fEPSPs) and paired-pulse facilitation (PPF; 50- and 100-msec interstimulus intervals, ISIs) was investigated in the projection from hippocampal area CA1 to the subiculum. We also investigated whether LTD can be obtained in the subiculum using a stress protocol (previously described to induce LTD effectively in vivo in area CA1 of the hippocampus; Xu, Anwyl, & Rowan, 1997). Finally, we examined the interaction between the stress treatment and PPF. There was no significant difference between fEPSP amplitude measured at 30 min post-LFS (10 Hz) when compared with baseline fEPSP peak amplitude; PPF, however, increased significantly 30 min post-LFS when compared with PPF pre-LFS for both 50-msec and 100-msec intervals. These results indicate that there is a dissociation between single-pulse stimulation and paired-pulse stimulation of the CA1-subiculum pathway. The combined effect of stress and LFS produced a depression in synaptic response of 56.99% at 5 min post-LFS and 10.23% at 30 min post-LFS. Behavioral stress combined with LFS caused a significant decrease in PPF at 30 min poststimulation. At a 50-msec ISI, facilitation is minimal; at a 100-msec ISI, paired-pulse depression occurs. These data suggest that there possibly are previously undescribed mechanisms regulating transmission in this pathway.

Paired-pulse facilitation (PPF) is the phenomenon whereby the field excitatory postsynaptic potential (fEPSP) responses to a second stimulus is enhanced relative to the first, if the second stimulus is delivered within a relatively brief period of time after the first (Zucker, 1989). The effect is thought to be primarily presynaptic, resulting from a transient increase in calcium levels in the presynaptic terminal. PPF is thus a short-term (milliseconds) strengthening of synaptic transmission. In contrast, long-term potentiation (LTP) is a long-term (hours to weeks) strengthening of synaptic transmission (Bliss & Collingridge, 1993; Bliss & Lomo, 1973). LTP is a popular model for some of the biological processes that may be engaged during biological consolidation of memories (Maren & Baudry, 1995). LTP was first described in the hippocampus (Bliss & Lomo, 1973), a structure vital for

learning and memory (Rolls & O'Mara, 1993). The locus of change after LTP induction has been the subject of much debate; it is still a matter of controversy whether LTP is primarily a presynaptic or a postsynaptic phenomenon or some combination of the two (Bliss & Collingridge, 1993). One method of determining the extent to which presynaptic or postsynaptic factors predominate in LTP is to examine the interaction between PPF and LTP. We have previously described PPF in the projection from hippocampal area CA1 to the subiculum (Commings, Gigg, Anderson, & O'Mara, 1998b) and have examined the interaction between PPF and LTP. The amount of facilitation decreases significantly when examined 30 min after the induction of LTP, suggesting the possibility of presynaptic involvement in LTP maintenance.

We have also found (Anderson, Commings, & O'Mara, 2000) that stimulation of the CA1-subiculum pathway with low-frequency stimulation (LFS; 10 Hz, 900 pulses) does not lead to long-term depression (LTD) of synaptic transmission. It is currently unknown whether there is some interaction between such LFS treatments and PPF. Typically, LTD has been difficult to induce in vivo (Doyere, Errington, Laroche, & Bliss, 1996). One method that does successfully induce LTD effectively in vivo in hippocampal area CA1 is to behaviorally stress the animal for

This work was supported by the Wellcome Trust, the European Commission, and the Arts and Social Sciences Benefaction Fund & Provost's Fund of Trinity College. The authors thank Marina Lynch (Department of Physiology, Trinity College) and Colin Gemmell and Michael Anderson (Department of Psychology, Trinity College) for helpful discussions. Correspondence should be addressed to S. M. O'Mara, Department of Psychology, University of Dublin, Trinity College, Dublin 2, Ireland (e-mail: smomara@tcd.ie).

30 min prior to the commencement of the experiment (Xu, Anwyl, & Rowan, 1997). In the present study, we examined whether there is an interaction between LFS and PPF. We also examined whether robust LTD can be obtained using a stress protocol in the subiculum. Finally, we examined whether there is an interaction between the stress treatment and PPF. Induction of LTD might significantly increase facilitation as a result of a decrease in the level of baseline synaptic transmission. This is based on the idea that LTP, which increases synaptic efficacy, causes a reduction in PPF post-LTP induction (Commins et al., 1998b; Schulz, Cook, & Johnston, 1994). LTD, which decreases synaptic efficacy (Dudek & Bear, 1992), opposing LTP, would therefore cause an increase in facilitation post-LTD induction. The goal of this study was to throw some light on the possible mechanisms of LTD induction and maintenance, provide further explanations of presynaptic mechanisms and, finally, provide insights into the relationships between the behavioral state of the animal and synaptic transmission.

METHOD

Adult male Wistar rats (weight: 200–300 g) were initially anesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and were mounted in a stereotaxic holder. Further injections of urethane (ethyl carbamate: 1.5 g/kg, i.p.) were given to sustain anesthesia throughout the experiments. A local anesthetic/adrenaline combination was injected under the scalp and an incision was made such that the skull was visible. Stimulating electrodes were aimed at area CA1, and the recording electrodes were aimed at the dorsal subiculum. This is a monosynaptic projection, as confirmed by our own dual-stimulation experiments in which we have examined both single-unit and fEPSP responses (Gigg, Finch, & O'Mara, 2000) and other data (Finch, Tan, & Isokawa-Akesson, 1988). Electrode implantation sites were identified using stereotaxic coordinates, relative to bregma (Paxinos & Watson, 1986); recording electrodes were 6.8 mm posterior and 4.0 mm lateral to the midline, and stimulating electrodes were 4.5 mm posterior and 2.5 mm lateral to the midline. Bipolar stimulating and monopolar recording electrodes consisted of two pieces of twisted 50- μ m tungsten wire, insulated to the tips.

Signals were filtered between 0.1 Hz and 1 KHz and then amplified. Recordings were digitized on line using a PC connected to a CED-1401 plus interface (CED, Cambridge). Signals were also monitored using an oscilloscope. Electrodes were slowly lowered to a depth of 2.5 mm; test pulses were administered during electrode movement at a rate of 0.1 Hz (see Commins, Gigg, Anderson, & O'Mara, 1998a). The final depths were adjusted until maximal fEPSPs were obtained; electrodes were allowed to settle for 10 min before baseline recordings were conducted. Initially, input/output (I/O) analyses were conducted to determine the maximal response to constant increments in stimulus intensity. Baseline measurements were conducted at an intensity to produce half-maximal fEPSPs for a minimum of 15 min. The n in each experiment described refers to the numbers of animals used. For the purpose of data analysis, we measured fEPSP amplitude, which is expressed as the difference between the peak and baseline, measured in millivolts. We recorded fEPSP amplitude rather than fEPSP slope because we have previously found (see Commins et al., 1998a) that fEPSP amplitude is a more conservative and less variable measure than is slope in this projection. Unless stated otherwise, all data are expressed as percentage mean $\pm$ SEM of baseline fEPSP. We conducted a series of one-way analyses of variance (ANOVAs) with the appropriate post hoc test (Newman-Keuls at the 5% level of signif-

icance) when comparing more than two groups. Otherwise, we used paired t tests (5% and 1% levels of significance indicated where appropriate with * and **, respectively).

Paired-Pulse Facilitation Protocols

Paired-pulse effects were examined after the baseline was established for a minimum of 10 min. Pairs of stimuli were delivered every 20 sec with 50- and 100-msec ISIs. The first pulse and second pulse elicited by the first and second stimulus of the stimulus pair will be referred to as fEPSP1 and fEPSP2, respectively. The PPF value was calculated by taking the average of six peak amplitude values of fEPSP1, for a given ISI, and normalizing the average of six values for fEPSP2 with respect to this value in percentage terms.

Examining the Effect of PPF With Time

A baseline was initially established with stimulation at half-maximal peak fEPSP amplitude for 10 min, after which PPF was measured. ISIs of 50 and 100 msec were used. Baseline stimulation was resumed for a further 30 min when PPF was again measured at the two ISIs.

Testing the Interaction Between LFS and PPF

Again, a baseline was initially established with stimulation at half-maximal peak fEPSP amplitude for 10 min, after which PPF was measured. ISIs of 50 and 100 msec were again used. Baseline stimulation was then resumed for a further 5 min. The induction of LTD was then attempted by LFS (900 pulses at 10 Hz and with stimulus intensity during LFS set at baseline intensity). Baseline stimulation was then resumed at 0.1 Hz for a further 30 min. PPF was subsequently measured at 30 min post-LFS at the two ISIs.

Testing for the Ability to Induce LTD in Stressed Animals and Examining the Interaction With PPF

The animals were stressed by taking them out of their home cage in which they were housed with other rats and were placed, prior to anesthetization, for 30 min into a novel, yellow, empty box (25 $\times$ 25 $\times$ 30 cm) that was elevated and brightly lit. Defecation, "freezing" behavior, and piloerection indicated that the animals were stressed. This stress induction protocol was similar to that used by Xu, Anwyl, and Rowan (1997). The animals were then anesthetized, and baseline stimulation was started for 10 min. The effects of paired-pulse stimulation was then measured. ISIs of 50 and 100 msec were again used. Baseline stimulation was resumed for a further 5 min. The induction of LTD was then attempted by LFS (900 pulses at 10 Hz). Baseline stimulation was then resumed at 0.1 Hz for a further 30 min. PPF was again measured at 30 min post-LFS at the two ISIs.

Histological Processing

After each experiment, the rats were overdosed with sodium pentobarbitone, and their brains removed and allowed to sink in 4% formaldehyde. All brains were then examined to verify the position of the stimulating and recording electrodes. All data presented here are for stimulating and recording sites that were verified as being in CA1 and subiculum, respectively. Figure 1 provides details of stimulating and recording sites in CA1 and subiculum, respectively. It can be seen that, for the most part, the stimulating electrode is placed centrally in CA1 in stratum oriens and stratum pyramidale, and the recording electrode is placed dorsal to middle portion of the longitudinal (septotemporal) axis of the subiculum.

RESULTS

PPF Does Not Change With Time

A baseline was established ($n = 6$) with stimulation at half-maximal peak fEPSP amplitude. PPF was measured 10 min into the baseline for ISIs of 50 and 100 msec. For

Figure 1. Examples of stimulating and recording sites in CA1 and subiculum, respectively.

the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was $29.47\% \pm 3.7\%$; for the 100-msec ISI, the percentage facilitation was less at $25.56\% \pm 3.6\%$ (see Figure 2b, -5 min). Baseline stimulation was then resumed for approximately 30 min, and this remained relatively stable as demonstrated by the fEPSP amplitude measure which stood at $97.45\% \pm 5.8\%$ of baseline at the end of the recording period (see Figure 2a). A one-way ANOVA was then conducted to compare the first 5 min, the middle 5 min, and the final 5 min of the recording period. As expected, the overall result was not significant [$F(2,77) = 1.8963, p > .1$]; subsequent post hoc tests (Student Newman-Keuls) revealed no significant differences between the three time periods, confirming the stability of fEPSPs.

PPF was again measured at the end of the recording period (see Figure 2b, 30 min) for the two intervals. For the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was found to be $31.45\% \pm 4.7\%$; for the 100-msec ISI, the percentage facilitation was again less at $29.83\% \pm 4.6\%$. A paired-sample t test was used to compare PPF at both 50-msec and 100-msec ISIs. PPF

measured at 10 min into the recording period was compared with PPF measured at the end of the recording period. For the 50-msec ISI, no significant difference was found between the PPF values measured [$t(35) = 0.61, p > .05$]. For the 100-msec ISI, there was also no significant difference between PPF at the start of the recording period and PPF measured at the end of the recording period [$t(35) = 1.27, p > .05$]. Representative examples of fEPSPs at various points of the recording periods are presented in Figure 2a (inset). The fEPSPs displaying PPF, for both 50-msec and 100-msec ISIs, are presented in Figure 2c at both the start and the end of the recording period.

Interaction Between LFS (10 Hz) and PPF

A baseline was initially established ($n = 6$) with stimulation at half-maximal peak fEPSP amplitude. PPF was measured at 10 min into the baseline for the 50-msec and 100-msec ISIs. For the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was $31.41\% \pm 2.8\%$; for the 100-msec ISI, the percentage facilitation was less at $23.36\% \pm 1.7\%$ (see Figure 3b, pre-LFS). Baseline stimulation was then resumed for approximately

Figure 2. (a) Baseline responses show little fluctuation over time. Sample field excitatory postsynaptic potential (fEPSP) traces displayed above the plot, with numbers corresponding to the time in which the trace was taken. (b) A bar chart showing percentage paired-pulse facilitation (PPF) at the two time periods (-5 min and 30 min), taken at the respective points in panel a. ISIs of 5 and 100 msec were used. (c) Examples of fEPSPs displaying PPF for both ISIs (the right-hand traces are taken at the end of the recording period). Calibration bars represent 0.5 mV and 5 msec.

5 min. The induction of LTD was then attempted by LFS. Baseline stimulation was again resumed at 0.1 Hz. Initially, there was a depression in synaptic response, demonstrated by an fEPSP amplitude that stood at $68.56\% \pm 10\%$ of baseline 5 min post-LFS. The response gradually increased back to baseline levels that stood at $102.42\% \pm$

3.61% 30 min post-LFS (see Figure 3a). Representative examples of fEPSPs pre- and post-LFS are also presented in Figure 3a (inset).

A one-way ANOVA was then conducted to compare the baseline period, 0 – 5 min post-LFS, and the final 5 min of the recording period. The overall result was significant

Figure 3. (a) Effect of low-frequency stimulation (LFS; 10 Hz) on the amplitude of field excitatory postsynaptic potentials (fEPSPs). The post-LFS values are expressed as percentage of the prestimulation baseline $\pm$ SEM. Sample fEPSP traces are displayed above the plot with numbers corresponding to the time where the traces were taken. (b) A bar chart showing percentage paired-pulse facilitation (PPF) both pre- and post-LFS for ISIs of 50 and 100 msec. Note the increase in facilitation at both ISIs. (c) Examples of fEPSPs displaying PPF for both ISIs (the right-hand traces are post-LFS). Calibration bars represent 0.5 mV and 5 msec. ** $p < .01$.

[$F(2,136) = 427.14$, $p < .001$], and subsequent post hoc tests (Student Newman-Keuls, $p < .05$) revealed a significant decrease in the response 5 min post-LFS when compared with the baseline period. There was also a significant

difference between the 5-min period post-LFS and the final 5 min of the recording period. However, there was no difference between the baseline period and the last 5 min of the recording period, indicating that fEPSP am-

plitude had returned completely to baseline levels over the 30-min recording period.

PPF was again measured at the end of the recording period (see Figure 3a) for the two intervals. For the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was $44.99\% \pm 5.3\%$; for the 100-msec ISI, the percentage facilitation was $38.88\% \pm 4.8\%$. A paired-sample *t* test was used to compare PPF at both 50-msec and 100-msec ISIs. PPF measured at 5 min pre-LFS was compared with PPF measured at 30 min post-LFS. For the 50-msec ISI, a significant increase in facilitation was found at 30 min post-LFS when compared with PPF values measured pre-LFS [$t(35) = 3.28, p < .01$]. For the 100-msec ISI, there was also a significant increase in facilitation post-LFS when compared with PPF measured 5 min pre-LFS [$t(35) = 3.00, p < .01$]. There was a significant increase in facilitation post-LFS at both the 50-msec and 100-msec ISIs (Figure 3b). These results indicate that, although the fEPSP response clearly returns to baseline levels, suggesting that the LFS at 10 Hz has no effect in this pathway, the significant increases in PPF post-LFS suggest the opposite: LFS has a facilitatory effect on PPF. The fEPSPs displaying PPF, for both 50-msec and 100-msec ISIs (pre- and post-LFS) are presented in Figure 3c.

Interaction Between Stress and LFS

The animals were stressed for 30 min prior to anesthesia. A baseline was initially established ($n = 5$) with stimulation at half-maximal peak amplitude. PPF was measured at 10 min into the baseline for the 50- and 100-msec ISIs. For the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was $49.74\% \pm 4.03\%$; for the 100-msec ISI, the percentage facilitation was at $14.16\% \pm 2.2\%$ (see Figure 4b, pre-LFS). The baseline was then resumed for approximately 5 min. The induction of LTD was then attempted by LFS (900 pulses at 10 Hz). Baseline stimulation was again resumed at 0.1 Hz. Initially, there was a depression in synaptic response; fEPSP amplitude stood at $43.01\% \pm 13.4\%$ of baseline 5 min post-LFS. The response gradually increased; however, at the end of the 30-min recording period, it still had not returned to baseline levels, suggesting a moderate level of LTD present at 30 min. The fEPSP amplitude stood at $89.77\% \pm 13.1\%$ of baseline 30 min post-LFS (see Figure 4a). Representative examples of fEPSPs pre- and post-LFS are also presented in Figure 4a (inset).

A one-way ANOVA was then conducted to compare the baseline period, 0–5 min post-LFS, and the final 5 min of the recording period. The overall result was significant [$F(2,117) = 969.73, p < .001$]; subsequent post hoc tests (Student Newman–Keuls, $p < .05$) revealed a significant decrease in the response 5 min post-LFS when compared with the baseline period and also between the 5-min period post-LFS and the final 5 min of the recording period. There was also a significant decrease in response of the last 5 min of the recording period when

compared with baseline, confirming depression in the synaptic response 30 min post-LFS.

PPF was again measured at the end of the recording period (see Figure 4a) for the two intervals. For the 50-msec ISI, the percentage increase of the second fEPSP relative to the first was $3.89\% \pm 5.49\%$. For the 100-msec ISI, there was evidence of paired-pulse depression (PPD); the percentage depression was found to be $20.32\% \pm 6.65\%$ below baseline. A paired-sample *t* test was used to compare PPF at both 50-msec and 100-msec ISIs. PPF measured at 5 min pre-LFS was compared with PPF measured at 30 min post-LFS. For the 50-msec ISI, a significant decrease in facilitation was found at 30 min post-LFS when compared with PPF values measured pre-LFS [$t(29) = -5.48, p < .01$]. For the 100-msec ISI, there was also a significant decrease in facilitation post-LFS when compared with PPF measured 5 min pre-LFS to the extent that PPD was evident [$t(29) = -5.16, p < .01$]. There was a significant decrease in facilitation post-LFS at both 50-msec and 100-msec ISIs (to the point that there was depression at the 100-msec ISI post-LFS; Figure 4b). These results indicate that depression of the synaptic response brought about by the interaction between stress and 10-Hz LFS has a substantial effect in the CA1–subiculum pathway. The fEPSPs displaying PPF, for both 50-msec and 100-msec ISIs, pre- and post-LFS are presented in Figure 4c.

Comparison of fEPSP1 and fEPSP2

To examine the interaction between LFS and PPF and investigate the underlying causes of the changes in PPF—that is, the increase in PPF following LFS and the decrease in PPF following LFS combined with stress—we compared the average peak amplitude of fEPSP1 both pre- and post-LFS. We also compared the average fEPSP peak amplitude of the second fEPSP both pre- and post-LFS. We investigated this at both 50-msec and 100-msec ISIs. Figures 5a and 5b demonstrate the effect of 10-Hz LFS on both fEPSP1 and fEPSP2. The second fEPSP amplitude increased after LFS, and this effect was significant at the 100-msec ISI [paired-sample *t* test: 50-msec ISI, $t(29) = -1.47, p > .05$; 100-msec ISI, $t(29) = -2.47, p < .05$]. These results partially explain the increases in PPF following LFS at 10 Hz when compared with baseline PPF.

Combining behavioral stress and LFS also had a significant effect on PPF 30 min post-LFS. For the 50-msec ISI, facilitation was very much reduced when compared with baseline PPF. For the 100-msec ISI, there was a depression in the paired-pulse response. We then compared the average peak amplitude of fEPSP1 both pre- and post-LFS. The average fEPSP peak amplitude of the second fEPSP was also compared in the same manner. Both 50-msec and 100-msec ISIs were used. Figures 5c and 5d show a significant increase in fEPSP1 at both 50-msec ISI and 100-msec ISI post-LFS when compared with baseline PPF [paired-sample *t* test: 50-msec ISI, $t(29) = -4.55, p < .01$; 100-msec ISI, $t(29) = -5.52, p < .01$].

Figure 4. (a) Effect of stress and low-frequency stimulation (LFS; 10 Hz) on the amplitude of field excitatory postsynaptic potentials (fEPSPs). Sample fEPSP traces are displayed above the plot, with numbers corresponding to the time in which the traces were taken. (b) A bar chart showing percentage paired-pulse facilitation (PPF) both pre- and post-LFS for ISIs of 50 and 100 msec. Note that, at 50-msec ISI, facilitation is minimal post-LFS; at 100-msec ISI, paired-pulse depression (PPD) is evident. (c) Examples of fEPSPs displaying PPF or PPD for both ISIs (the right-hand traces are post-LFS). Calibration bars represent 0.5 mV and 5 msec. ** $p < .01$.

At the same time, there was a significant decrease in the second fEPSP at both levels post-LFS when compared with baseline PPF [paired-sample t test: 50-msec ISI, $t(29) = 2.54$, $p < .05$; 100-msec ISI, $t(29) = 2.49$, $p < .05$].

Again, these results may explain the decrease in PPF post-LFS when compared with baseline PPF following LFS combined with stress—that is, there was a significant increase in fEPSP1.

Figure 5. (a) A bar chart comparing both fEPSP1 and fEPSP2 pre-LFS with fEPSP1 and fEPSP2 post-LFS at the 50-msec ISI. (b) A bar chart comparing both fEPSP1 and fEPSP2 pre-LFS with fEPSP1 and fEPSP2 post-LFS at the 100-msec ISI. (c) A bar chart comparing both fEPSP1 and fEPSP2 pre-LFS with fEPSP1 and fEPSP2 post-LFS at the 50-msec ISI with the added stress factor. (d) A bar chart comparing both fEPSP1 and fEPSP2 pre-LFS with fEPSP1 and fEPSP2 post-LFS at the 100-msec ISI with the added stress factor. * $p < .05$. ** $p < .01$.

DISCUSSION

In this study, we examined the interactions between LFS, stress, and PPF. We found that, although LFS had no effect on baseline synaptic transmission, it did significantly increase PPF. Thus, baseline synaptic transmission and PPF of the same pathway can be dissociated. Further-

more, we found that behavioral stress in association with LFS induced significant levels of depression in baseline synaptic transmission and also induced PPD of synaptic transmission. Control experiments demonstrated that these changes were not a result of the passage of time.

It has been historically difficult to obtain LTD in vivo, despite the use of standard protocols that have been suc-

cessful in inducing LTD in vitro (Dudek & Bear, 1992). The difficulty in obtaining LTD is not confined to "early" components of the hippocampal circuitry, such as the dentate gyrus (Doyere et al., 1996); we were also unable to induce LTD in the subiculum (Anderson et al., 2000). Although there are no changes in baseline synaptic transmission resulting from this protocol, underlying changes do in fact occur: PPF following 10-Hz LFS was significantly increased, although baseline synaptic transmission recovered rapidly, stabilizing at baseline levels by 30 min post-LFS. Why does this extra facilitation above baseline PPF levels occur? We compared the first fEPSP of the paired-pulse pre-LFS with the first response post-LFS; we also compared the second fEPSP pre-LFS with that post-LFS. The second fEPSP increased significantly post-LFS, suggesting that there are underlying changes that are not obvious from examining baseline synaptic transmission—a *metaplastic effect* (Abraham & Bear, 1996).

The Interaction Between Stress and LFS

Xu, Anwyl, and Rowan (1997) have shown that exposure to stress for 30 min prior to the commencement of the experiment is effective in inducing LTD in vivo in hippocampal area CA1. We examined for the first time the effect of stress combined with LFS in the CA1–subiculum pathway and found that the addition of stress causes a depression in synaptic response to $89.77\% \pm 13.1\%$ of baseline when measured 30 min post-LFS. LFS of 10 Hz alone had no effect, but the addition of behavioral stress had a significant effect. The magnitude of depression is somewhat less in the subiculum than that found in area CA1; Xu, Holscher, Anwyl, and Rowan (1998), for example, reported a depression to 78.9% of baseline using LFS (900 pulses at 3 Hz) combined with behavioral stress. Although stress is known to affect hippocampal synaptic plasticity, little is known about how it does so (Xu et al., 1998). Here, by examining PPF both pre- and post-LFS, we hope to provide some insight into how stress combined with LFS affects the regulation of synaptic transmission. Our experiments were conducted on anesthetized animals; other experiments have been conducted under different anesthetic conditions or without anesthetic, for example, in the freely moving animal (Diamond, Fleshner, & Rose, 1994; Xu, Holscher, Anwyl, & Rowan, 1997). These differing studies suggest that the effects of behavioral stress are extremely powerful and are independent of the anesthetized state of the animal.

We had predicted that a depression in synaptic efficacy would cause a significant increase in facilitation (see introduction). This was based on the hypothesis that LTP, which increases synaptic efficacy and reduces PPF post-LTP induction (Schulz et al., 1994), would oppose the effect of LTD, which decreases synaptic efficacy, causing an increase in facilitation post-LTD induction. In a recent study in which the relationship between PPF and LTD induced by intracellular tetanization of postsynaptic neurons was examined, Volgushev, Voronin, Chistakova, and Singer (1997) found that, in inputs that under-

went LTD, the PPF ratio increased during the initial 30 min after tetanization. However, we found the opposite to occur when LFS is combined with stress. Depression of synaptic transmission induced by stress and LFS causes a significant decrease in PPF at 30 min poststimulation: At 50-msec ISI, facilitation is minimal; at 100-msec ISI, PPD occurs. We therefore compared the average peak amplitude of the first fEPSP pre-LFS to the average peak amplitude of the first fEPSP post-LFS. We also examined the second fEPSP pre- and post-LFS. The average peak amplitude value of the first fEPSP increased significantly at the 50- and 100-msec ISIs, and, at the same time, the second fEPSP decreased significantly again at the 50- and 100-msec ISIs post-LFS. Thus, we found that the nature of PPF was changed: The first fEPSP increases in amplitude, and the second fEPSP decreases in amplitude.

PPF and Time

The changes that we have observed above are not related to time. We found there were no significant differences in PPF at the end of the recording period when compared with PPF measured at the start in control animals. This is true for both ISIs tested. This is an important control, suggesting that any changes that occur in PPF post-intervention are in fact due to the intervention rather than a time-related phenomenon.

Mechanism of Action

PPF is attributed to the persistence of residual calcium in the presynaptic terminal, which causes an enhanced release in response of the second fEPSP (Hess & Kund, 1992; Katz & Miledi, 1968; Volgushev et al., 1997; Zucker, 1989). PPF ratios are thought to reflect the functional state of presynaptic release mechanisms, whereby high PPF ratios are indicative of a low release probability and low PPF ratios represent a high release probability (Zucker, 1989).

The degree or direction of synaptic change induced by a particular pattern of stimulation cannot be predicted unless the previous stimulation history of the tissue is known (Abraham & Bear, 1996). Our results give credence to this suggestion: We found that different patterns of stimulation and different behavioral states of an animal have differential effects on synaptic transmission. The potential of synapses to undergo potentiation or depression depends on the actual state of presynaptic release mechanisms: Inputs with an initially low release probability (displaying high PPF) have a tendency to become potentiated, whereas inputs with a high initial release probability (displaying low PPF) have a tendency to become depressed (Volgushev et al., 1997). Induction of LTP causes an increase in release probability (Schulz et al., 1994; Sokolov, Rossokhin, Behnisch, Reymann, & Voronin, 1998), thus enhancing the probability of obtaining LTD. Indeed, it has been shown that LTP induction facilitates subsequent LTD induction (depotentialization; Bashir & Collingridge, 1994; Fujii, Saito, Miyakawa, Ito, & Kato, 1991). Synaptic stimulation has the effect of changing the

functional regulation of synaptic transmission, whereby the probability of release is enhanced or reduced (the magnitude and direction of these changes probably depend on the initial state of the synaptic terminal). The synapses in the subiculum show PPF and a low probability of release; when these synapses are subjected to 10-Hz LFS, they undergo a metaplastic change. The synapses post-LFS show an increase in PPF, suggesting that they now have a lower probability of release when compared to a naive pathway. Thus, we predict that these synapses post-LFS are more susceptible to the induction of LTP. However, there are other alternative explanations to alterations in the probability of transmitter release. There may be a change in the number of active release sites following LFS or indeed a combination of a change in the number of active sites with a change in the probability of release (see Schulz et al., 1994, and Volgushev et al., 1997, for details) or other changes in postsynaptic mechanisms (Wang & Kelly, 1997). We suggest that changes in inhibition had little, if any, influence on our results. Schulz, Cook, and Johnston (1995) found that PPF is not affected by GABA_A- or GABA_B-mediated inhibition and that PPF changes persist with both GABA_A and GABA_B antagonists. This is confirmed in a more recent study by Sokolov et al. (1998). Furthermore, although inhibitory synaptic transmission can undergo use-dependent change (Komatsu, 1994), LTP and LTD are believed to reflect mainly changes in excitatory synaptic transmission (Bliss & Collingridge, 1993). Finally, a smooth decay of our fEPSPs suggests that there was no inhibition. The depressive effect on PPF is caused by a significant increase in fEPSP1, consistent with the view that PPD is observed if the first action potential successfully triggers transmitter release at most release sites (Debanne, Guerineau, Gähwiler, & Thompson, 1996). The decreasing effect of fEPSP2 after stress and LFS could be explained by the fact that fEPSP1 increased toward the maximal response. PPF in CA1 is known to change into PPD when high stimulation intensities are used (Ghijsen & Lopes da Silva, 1991).

Stress causes profound changes in the brain (Lupien et al., 1998). We have found a significant reduction in PPF post-LFS combined with stress to the extent that facilitation is minimal at some ISIs and PPD is observed at other ISIs. It is possible that stress affects voltage-gated L-type calcium channels and causes excess calcium entry (Xu, Anwyl, & Rowan, 1997), which is reflected in the increase in the first fEPSP of the pair, as we have observed. This may not only cause a reduction in PPF but may also lead to LTD. The response to the second stimulus is dependent on what happens during the first. If transmitter release does not occur on the first stimulus, the probability of release on the second is greatly enhanced, and the opposite is also true (Lisman, 1997). We suggest that the effects of LFS on the synapses alone are masked by stress, which causes a depletion of readily releasable vesicles that are not replenished quickly.

Stress may have the effect of decreasing the signal-to-noise ratio of synaptic transmission, which in turn decreases synaptic efficacy. Therefore, there will be in turn an effect on the processing and storage of information, especially in structures that are susceptible to stress, such as the hippocampal formation. Future behavioral experiments should examine the interaction between tasks that are dependent on the integrity of structures that have high densities of glucocorticoid receptors and those that are dependent on the integrity of structures that have high levels of chronic or acute behavioral stress. Furthermore, examination of the interactions between the blockade of corticosterone release and LFS in this pathway would provide further insight into the mechanisms underlying behavioral stress in the subiculum.

Conclusion

We conclude that baseline synaptic transmission and PPF of the CA1-subiculum pathway can be dissociated; LFS had no effect on baseline synaptic transmission but significantly increased PPF. Behavioral stress induces depression in baseline synaptic transmission in the same pathway and also decreases PPF, dramatically inducing PPD at the 100-msec ISI. Possible mechanisms involving a presynaptic component to LFS were discussed in light of these results using novel analytic methods.

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(Manuscript received June 10, 1999;
revision accepted for publication October 16, 1999.)