Very short-term plasticity in hippocampal synapses

Lynn E. Dobrunz, Emily P. Huang, and Charles F. Stevens*

Molecular Neurobiology Laboratory and Howard Hughes Medical Institute, Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037

ABSTRACT Hippocampal pyramidal neurons often fire in bursts of action potentials with short interspike intervals (2–10 msec). These high-frequency bursts may play a critical role in the functional behavior of hippocampal neurons, but synaptic plasticity at such short times has not been carefully studied. To study synaptic modulation at very short time intervals, we applied pairs of stimuli with interpulse intervals ranging from 7 to 50 msec to CA1 synapses isolated by the method of minimal stimulation in hippocampal slices. We have identified three components of short-term paired-pulse modulation, including (*i*) a form of synaptic depression manifested after a prior exocytotic event, (*ii*) a form of synaptic depression that does not depend on a prior exocytotic event and that we postulate is based on inactivation of presynaptic N-type Ca^{2+} channels, and (*iii*) a dependence of paired-pulse facilitation on the exocytotic history of the synapse.

In vivo single-unit recordings show hippocampal pyramidal cells commonly fire in a characteristic pattern called a complex spike (1, 2). Complex spikes are brief bursts of two to nine action potentials with interspike intervals ranging from 2 to 10 msec (3, 4). Several lines of evidence suggest that these action potential bursts play an important role in hippocampal function. For example, bursts of stimuli similar to the complex spike effectively induce long-term potentiation in hippocampal synapses when delivered at the frequency of the θ rhythm (5–10 Hz), a naturally occurring oscillation seen in the hippocampal electroencephalogram (5). In fact, a single such burst is capable of inducing robust long-term potentiation in hippocampal slice when given at the peak of a cholinergically induced θ rhythm (6).

Behavioral studies also point to the importance of pyramidal cell bursting. Hippocampal pyramidal cells are known to act as "place cells"; that is, their firing rate depends on the location of the animal within the environment. The area occupied by the animal when one observes increased firing in a particular cell is called that cell's "place field." Although the place field of a given cell is typically mapped by measuring changes in its overall firing rate, Otto *et al.* (7) found that the field is more tightly defined when measured as an increase in the bursting rate. This result suggests that bursts may be the relevant activity in hippocampal spatial function.

Given that bursts with interspike intervals of 2–10 msec may be particularly relevant to the functional behavior of hippocampal neurons, it is notable that little is known about synaptic modulation at such short time periods. In particular, paired-pulse modulation at interpulse intervals of less than 20 msec should be carefully studied. Stevens and Wang (8) did examine paired-pulse facilitation at these time intervals in putative single synapses, but the limited focus of their study may have presented an overly simplified view of synaptic behavior. We have extended their work by applying paired

pulses to single synapses in hippocampal slice isolated by the method of minimal stimulation; the pulse pairs had interpulse intervals ranging from 7 to 50 msec. Our results demonstrate a complex range of modulatory behavior at short time intervals, including two forms of depression and a dependence of facilitation on the release history of the synapse.

MATERIALS AND METHODS

Slice Preparation. Transverse hippocampal slices were prepared from Long Evans rats (ages postnatal days 11–18) as previously described (9). Briefly, brain slices (350–400 μm) were cut by a Vibratome while immersed in an ice-cold solution containing 120 mM NaCl, 3.5 mM KCl, 0.7 mM $CaCl_2$, 4.0 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose. Slices were stored submerged in room temperature (22°C) solution, described above, and bubbled with 95% O_2/5% CO_2 for at least 1.5 hr before recording.

Electrophysiology. Whole-cell recordings from CA1 pyramidal cells were performed as described (9). During recording, slices were perfused with a room-temperature solution containing 120 mM NaCl, 3.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose. The recording solution was bubbled with 95% O_2/5% CO_2, and 50 μM picrotoxin was added to block inhibitory responses. Recording pipettes (2–4 MΩ) were filled with a solution containing 170 mM potassium gluconate, 10 mM Hepes, 10 mM NaCl, 2 mM $MgCl_2$, 1.33 mM EGTA, 0.133 mM $CaCl_2$, 3.5 mM MgATP, and 1.0 mM GTP. Recording solution was diluted to 300–305 milliosmolar. After obtaining whole-cell recordings in CA1 cells, the Schaffer collateral pathway was stimulated with a tungsten bipolar electrode and recorded currents were digitally stored at 5–8 kHz.

Minimal Stimulation and Paired-Pulse Recordings. In the experiment described in Fig. 1, a moderate stimulus was used to excite a population of synapses. For most of the experiments in this study, however, the method of minimal stimulation (8, 10, 11) was used to isolate the response of a putative single synapse. The minimal stimulation method we use is described in detail in ref. 9. In brief, the stimulation intensity is lowered until the following criteria are met in the recorded synaptic responses: (*i*) the average response size and failure rate do not vary when stimulus intensity is varied ±5%, (*ii*) the latency and shape of the synaptic currents do not vary across trials, and (*iii*) turning the stimulation intensity down leads to an abrupt disappearance of all responses.

Pulse pairs with varying interpulse intervals (7, 11, 15, 19, 30, and 50 msec, or some subset of these) were applied at a repetition rate of 0.25 Hz (in some experiments, the repetition rate was different, either 0.1 or 2 Hz). Amplitude of first pulse response was measured directly; the amplitude of the second pulse response was measured by subtracting a template fitted to the first pulse response. For experiments performed at minimal stimulation, release probability for first and second pulses was calculated as one minus the failure rate of responses.

Abbreviation: PPR, paired-pulse ratio.
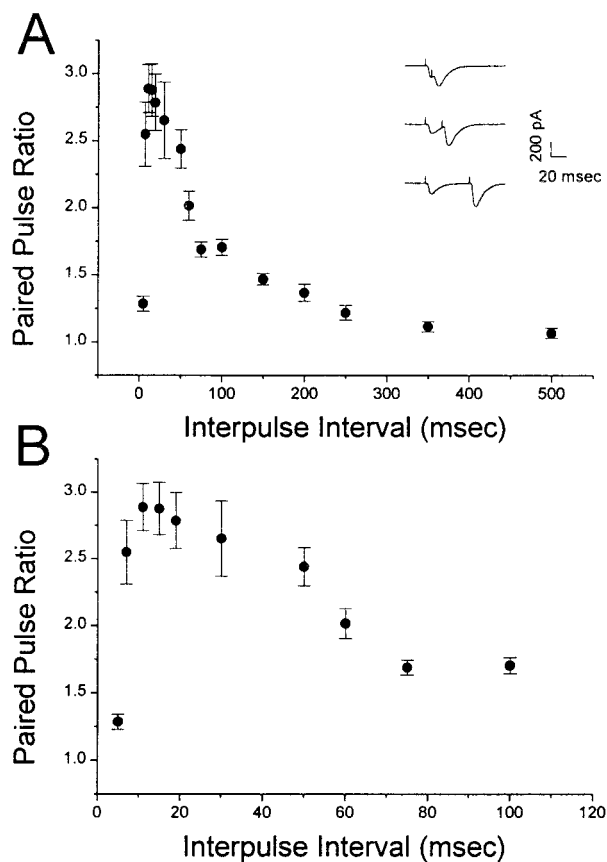*To whom reprint requests should be addressed. e-mail: cfs@salk.edu.

FIG. 1.   Paired-pulse modulation of macroscopic currents. (*A*) PPR plotted against interpulse interval, from 5 to 500 msec (each point is the mean ± SEM for 20–64 trials). (*Inset*) Sample traces from macroscopic current experiment. Shown (from top to bottom) are responses to paired pulses with interpulse interpulse intervals of 7, 19, and 50 msec, respectively. (*B*) Same plot as in *A*, with the *x* axis expanded to show detail at short time intervals.

RESULTS

We began by examining paired-pulse modulation of macroscopic currents recorded from a hippocampal CA1 pyramidal neuron. While recording from the cell, we stimulated its afferents with a sequence of paired pulses spaced at different interpulse intervals (see Fig. 1). The interpulse intervals ranged from 5 msec to 500 msec, from the average interspike interval observed in complex spikes *in vivo* (i.e., at 37°C) (7, 12) to the interval at which facilitation largely decays away (13).

We then calculated the paired-pulse ratio (PPR, the average response size on the second pulse divided by the average response size on the first) for each interpulse interval and plotted the results in Fig. 1. (Note that we measured the size of second pulse synaptic currents after correcting for contamination from first pulse synaptic currents.) From an interpulse interval of 5 msec, the PPR gradually increases to a peak at 15 msec, at which point the response is facilitated by a factor of 2.65; the PPR then shows a slow decay to resting levels at 500 msec.

This decay in facilitation ratio over a period of hundreds of msec has been extensively described and is attributed to the gradual removal of residual calcium from the presynaptic terminal (13). The fast rising phase of the ratio at short time intervals, however, has not been carefully studied; this phase could be a reflection of the time it takes for paired-pulse facilitation to develop or it could reflect very short-term mechanisms of depression superimposed on facilitation. One type of depression that acts at very short times has been described by Stevens and Wang (8), who examined paired-

pulse release at putative single synapses by using the method of minimal stimulation. Stevens and Wang (8) found that when synapses successfully release a quantum of neurotransmitter on the first of two pulses, they consistently exhibit depression on second pulses given at short time intervals. Synaptic release probability is completely depressed up to interpulse intervals of 5–7 msec and then recovers exponentially with a time course of about 4 msec. We term this form of depression "lateral inhibition" because we believe the first exocytotic event somehow increases the energy barrier for fusion of neighboring vesicles (see *discussion*).

Lateral inhibition, however, seems inadequate to explain the magnitude of depression observed in Fig. 1; the PPR at 5 msec measured as a fraction of peak PPR is 0.45. Because the average release probability of synapses is about 0.2 (11, 14), most synapses (~80%) would fail to release a quantum of neurotransmitter on the first pulse and would, therefore, not display lateral inhibition. If facilitation reaches its peak in <5 msec and no other source of depression were present, then one would expect to see macroscopically a depression of at most 20%; instead, we see that the depression is about 55%.

With the goal of discovering other potential sources of depression at short time intervals, we performed minimal stimulation experiments in hippocampal slice to study paired-pulse modulation at putative single synapses. For each synapse, we recorded the responses when we applied pairs of stimuli at interpulse intervals ranging from 7 msec to 19 or 50 msec. Fig. 2 shows sample excitatory postsynaptic currents obtained from these experiments.

Under conditions of minimal stimulation, frequent response failures are observed that reflect the probabilistic nature of synaptic transmitter release (8, 10, 11); the release probability (P) of a synapse observed by minimal stimulation is, therefore, one minus the failure rate. For 30 paired-pulse experiments, we calculated the initial release probability (P on the first pulse) and modulated release probability (P on the second pulse) at varying interpulse intervals. We were particularly interested, however, in how the presence of a release event on the first pulse would affect modulation of the second pulse, so we segregated our observations according to whether there was a release on the first pulse. Thus, for each interpulse interval, we calculated P_f, the release probability on the second pulse when there was a first pulse failure, and P_s, the release probability on the second pulse when there was a first pulse release.

Fig. 2 shows three representative synapses analyzed in this manner. These synapses were chosen because they clearly illustrate several phenomena that determine the way release probability behaves at short times after a first nerve impulse. In agreement with Stevens and Wang (8), all synapses expressed lateral inhibition, described above (see Fig. 2). As these examples demonstrate, P_s is generally close to zero at an interstimulus interval of 7 msec, reflecting dramatic depression of synaptic function after an exocytotic event; P_s then recovers exponentially, reaching its facilitated level by an interpulse interval of 20 msec. The average time constant of recovery in our experiments is 6.34 ± 3.45 msec (mean ± SD; $n = 20$).

The behavior of release probability after a first pulse failed to produce an exocytotic event varied widely between synapses (Fig. 2). Fig. 2*A* shows a synapse in which paired-pulse facilitation alone appears to dominate the release properties after a first pulse failure. At an interpulse interval of 7 msec, P_f has already reached the facilitated level at which it will remain till at least 20 msec. Also, in this synapse, P_f and P_s ultimately attain the same level of facilitation, so the only difference between the two states is the presence of lateral inhibition after a first pulse exocytotic event.

A significant number of synapses, however, display a marked decrease in release probability after a first pulse failure, a
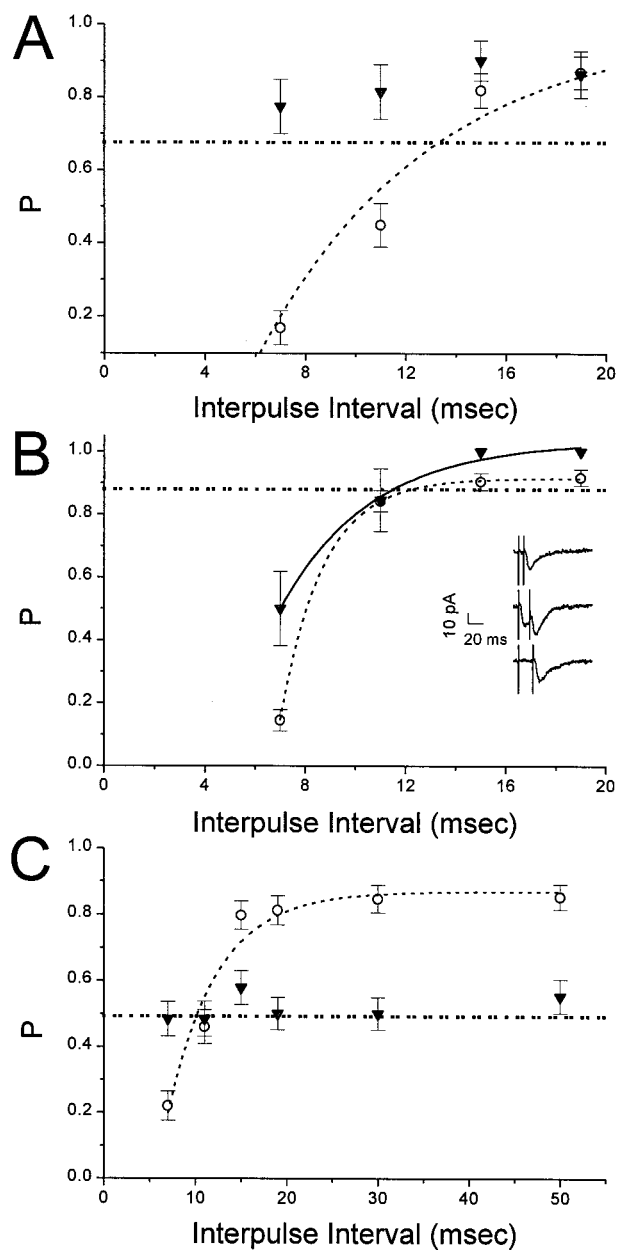
FIG. 2. Time course of release probability after synaptic use. (*A*) Release probability for second stimulus in a pair as a function of interpulse interval. Probabilities when the first stimulus did not result in an exocytotic event (P_f) are plotted as solid triangles {n for each point ranges from 27 to 31; error bars represent estimated SD, $[P(1 - P)/N]^{1/2}$}. Probabilities when the first stimulus did result in an exocytotic event (P_s) are plotted as open circles ($n = 59$ to 66). P_s as a function of time is fitted with an exponential decay (dashed line, recovery time constant = 7 msec), and the initial release probability of the synapse, indicated by the dotted line, was 0.675. In this synapse, P_s depresses markedly, illustrating the phenomenon of lateral inhibition; no other form of depression is evident. (*B*) As in *A*, for a synapse in which both P_f (solid triangles; $n = 12$ to 18) and P_s (open circles; $n = 110$ to 116) display depression; this synapse expresses inactivation as well as lateral inhibition. Both P_s and P_f are fitted with exponential decays, with recovery time constants of 1.7 (dashed line) and 3.6 msec (solid line), respectively, and the initial release probability (dotted line) was 0.88. (*Inset*) Sample traces from minimal stimulation experiments. Shown (from top to bottom) are responses to paired pulses with interpulse interpulse intervals of 7, 15, and 19 msec, respectively. Note the presence of release failures in the top and bottom traces. (*C*) As in *A*, for a synapse in which P_f (solid triangles; $n = 92$ to 106) does not facilitate, but P_s (open circles; $n = 78$ to 92) does, illustrating history-dependent facilitation. This synapse, like all synapses, displays lateral inhibition (but no inactivation) with a recovery time constant = 5.1 msec (dashed line); the initial release probability (dotted line) was 0.49.

phenomenon we term "release inactivation." Fig. 2*B*, for example, shows a synapse modulated by a component of depression in addition to that mediated by lateral inhibition. At an interpulse interval of 7 msec, P_f is depressed considerably below the initial release probability of the synapse. Like P_s, P_f recovers exponentially, reaching the same steady-state facilitated level as P_s by 20 msec. This observed component of depression cannot be attributed to lateral inhibition or vesicular depletion, because it occurs when the synapse did not release on the first pulse; neither can it be explained solely as a slow build-up of facilitation, because P_f at 7 msec depresses significantly below initial release probability.

In the above examples, the amount of steady-state paired-pulse facilitation appears to be equivalent whether or not the first pulse produced an exocytotic event. But in a number of synapses, we observed different degrees of facilitation when we compared P_f and P_s. Fig. 2*C*, for example, shows a synapse in which P_f fails to facilitate at all, while P_s facilitates considerably. We term this effect, in which the amount of facilitation differed depending on whether an exocytotic event did or did not occur, "history dependent facilitation." In other synapses displaying this type of modulation, P_f may facilitate relative to initial P but still facilitate less than P_s. Synapses that exhibit history dependent facilitation may or may not additionally express the inactivation seen in Fig. 2*B*.

From the above discussion, it is clear that several modulatory factors exist at short time intervals that may superimpose upon one another. To quantify the range of behavior seen in our experiments, we calculated the degree of inactivation and history dependent facilitation for each of the 30 cells in our study and plotted frequency histograms of these values (see Fig. 3). We define the degree of inactivation observed in a synapse as the ratio of P_f at 7 msec to P_f at 19 msec (in other words, P_f at short times as a fraction of the steady-state P_f). Thus, inactivation values near 1.0 mean that there is no decrease in release due to inactivation, and inactivation values close to zero represent synapses with nearly complete inactivation. Fig. 3*A* is the histogram plotting the occurrence frequency in our 30 cells of four ranges of inactivation. We see that about half the synapses have inactivation values of less than 0.3. The rest of the cells divide evenly among inactivation ranges 0.3 to 1.0. Five of the synapses (17%) show essentially no inactivation, like the synapse represented in Fig. 2*A*.

The history dependence of facilitation we define as the ratio of P_f at 19 msec to P_s at 19 msec (steady-state P when the first pulse fails as a fraction of steady-state P when the first pulse releases). A ratio near 1.0 indicates that the degree of facilitation does not depend on the release history of the synapse, and a ratio significantly different from 1.0 indicates the release history of the synapse influences the amount of facilitation it will express. Fig. 3*B* is the frequency histogram of facilitation ratios calculated in our 30 cells. The histogram shows that although a significant fraction of synapses show no history dependent facilitation, exactly half the synapses have a ratio less than 0.75. In these synapses, paired-pulse facilitation is significantly reduced when the first pulse fails to produce an exocytotic event (see Fig. 2*C*).

DISCUSSION

We have identified three components of paired-pulse modulation expressed at very short interstimulus intervals (less than 20 msec). One component, herein called "lateral inhibition," was described earlier by Stevens and Wang (8); the other two components were not described. Of these two latter components, the first is a form of depression that is seen even if the first pulse fails to produce an exocytotic event. We have called this type of modulation "release inactivation." The second component is a dependence of paired-pulse facilitation on the
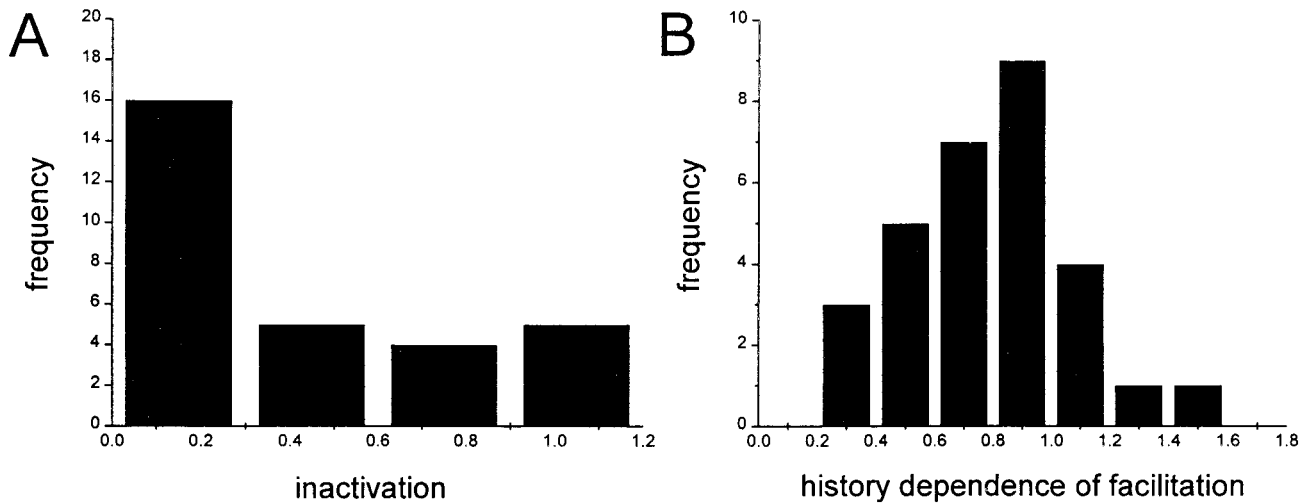
FIG. 3.   Frequency histograms for two forms of paired-pulse modulation. (*A*) Occurrence frequency for each range of inactivation values (P_f at 7 msec divided by P_f at 19 msec). Half of the synapses (16 of 30) show nearly complete inactivation (inactivation value < 0.3). (*B*) Occurrence frequency plotted against history dependence of facilitation (P_f at 19 msec divided by P_s at 19 msec). Nearly half of the synapses (13 of 30) show no history dependence in their facilitation (values between 0.8 and 1.2), and half the synapses (15 of 30) facilitate significantly less when the first pulse fails to release a quantum of neurotransmitter than when it does release one.

release history of the synapse ("history-dependent facilitation").

Lateral Inhibition. A previous study (8) presented a simple picture of paired-pulse modulation at short times: when the first pulse fails to release a quantum of neurotransmitter, release probability will facilitate in less than 5 msec to a steady-state level, and when the first pulse does release a vesicle, release probability will depress completely until 5–7 msec and then recover exponentially to the same steady-state level by 20 msec. We confirm that synapses in our study consistently show this latter form of depression (average recovery time constant = 6.34 msec), which might be explained by a number of possible mechanisms. One possibility is that each presynaptic terminal has only one release site that must be refilled after each release event before another can occur; the time course of refilling would correspond to the observed recovery from depression.

Another possible mechanism is that a given terminal has a number of release sites, each with a docked vesicle competent to fuse and release. When an influx of Ca^{2+} signals a release event, the probability that the vesicles will exocytose increases sharply, but if one of the vesicles does succeed in exocytosing, the energy barrier of fusion for the other docked vesicles increases so that they effectively cannot release. Synaptic release is then depressed until the fusion energy returns to its normal level. We favor this explanation of the release-dependent depression because it is consistent with the observation that release probability is approximately proportional to the measured size of the readily releasable pool (9), which we identify as the number of docked vesicles in a terminal (15). We therefore term this type of depression "lateral inhibition" (i.e., the release of one vesicle inhibits its neighbors from releasing).

Release Inactivation. Another form of short-term depression is evident, however, even when the first stimulating pulse does not release a vesicle. This type of depression is much more variable in degree of expression than lateral inhibition: some synapses do not display it at all, and in some synapses it rivals lateral inhibition in magnitude. Again, several possible mechanisms may underlie this type of depression. One likely explanation is that it is caused by the inactivation of presynaptic N-type calcium channels (16) after they have opened (or have been exposed to calcium) on the first pulse. We therefore have labeled this type of depression "inactivation." Because we have not measured the appropriate calcium currents, we cannot exclude other mechanisms for release inactivation, such

as a use-dependent modification of the exocytotic machinery. We can, however, argue against spike propagation failures as a mechanism; Stevens and Wang (8) measured the reliability of activating antidromic impulses with barely suprathreshold currents and found no failures of spike generation and propagation down to interpulse intervals <5 msec—i.e., less than the shortest interpulse interval used in our study.

History-Dependent Facilitation. Finally, we have identified heterogeneity in the amount of paired-pulse facilitation that a given synapse will express. Previous studies have identified heterogeneity in facilitation across synapses (9, 14); these studies show that synapses with low release probability facilitate much more than synapses with high release probability. Herein, we have found that the release history of a synapse also affects the amount of facilitation expressed. Specifically, about half the synapses in our study display significantly less facilitation when the first pulse fails to produce an exocytotic event than when it does produce one. A possible explanation is that a significant fraction of the release failures occur simply because action potentials fail to reach the terminal. Were this the case, no facilitation would be expected after such propagation failures because no calcium would enter the bouton. Allen and Stevens (11), however, measured the size of threshold fluctuations of the hippocampal presynaptic axons and found them to be less than about 5%. Because one of our criteria for acceptable minimal stimulation is that the release probability be invariant for a 5% change in stimulus intensity, we would generally exclude fibers whose threshold fluctuated enough to cause failures of stimulation. Also, Stevens and Wang (8) found no antidromic propagation failures when applying paired pulses at near threshold stimulation. Although we cannot exclude the possibility that orthodromic propagation might show different properties (for example, with respect to branch point failure), direct studies in culture (17) and indirect studies in slice (11) indicate this is not the case. Thus, it is unlikely that history-dependent facilitation is caused by failures associated with action potential propagation.

We favor an explanation based on the following observation: the amplitude of action potential produced Ca^{2+} transients in a fraction of presynaptic terminal show significant trial-to-trial variability (17). We propose that release failures are more likely to occur when the Ca^{2+} influx is smaller than average, particularly in synapses with low release probability, where there may be fewer docked vesicles to detect the Ca^{2+} signal (15). According to the residual Ca^{2+} model of facilitation (13),

the smaller the influx of Ca^{2+} on the first pulse, the less facilitation will be observed. Thus, looking at first pulse failures biases toward less Ca^{2+} influx and, therefore, less facilitation, and this effect will be more pronounced the lower the release probability of the synapse. Indeed, our measure of the history dependence of facilitation, the ratio of P_f to P_s at 19 msec, does seem to be a linear function of initial release probability; when the data are fit with a linear regression, the slope is 0.74 with a correlation coefficient of 0.65 ($n = 28$).

Reliability of Conclusions from Minimal Stimulation Experiments. Some questions about synaptic function require the study of single boutons, but the methods for doing this are imperfect. In the present situation, for example, none of the three described forms of depression could have been identified with experiments using populations of synapses. In fact, macroscopic experiments cannot even distinguish between short-term depressive mechanisms and a delayed development of facilitation. Stevens and Wang (8) placed statistical limits on the extent to which the minimal stimulation technique does provide data on just a single synapse, but in any particular instance, one can never be sure that one—and only one—synapse is being studied. For that reason, minimal stimulation data cannot be used to draw conclusions that critically depend on the inference one is studying single synapses. Our present conclusions about the three forms of early depression are not dependent on this assumption and are, therefore, robust to the possible presence of multiple synapses in our experiments.

We began our experiments by measuring paired-pulse modulation of macroscopic currents at short time intervals. How do these macroscopic observations relate to the processes we have identified herein? We noted that the magnitude of observed depression in the macroscopic PPR at 5 msec relative to that at 15 msec was unexpectedly large if lateral inhibition were the only mechanism of depression taken into account; we have identified a form of depression, inactivation, that may account for the difference. Both lateral inhibition and inactivation would depress the average synaptic current at very short times, but the relative contribution of each mechanism would depend on the average release probability of the synapses and also on the relationship between inactivation and release probability. This latter relationship has not yet been quantified, although we note a tendency in our experiments for synapses with higher P to show more depression due to inactivation. History-dependent facilitation further complicates the picture because it tends to oppose the above effects by flattening the time course of the PPR (there is less facilitation at long times). All

three of these effects are doubtlessly present in macroscopically measured paired-pulse modulation, but we do not now have enough information to identify the quantitative contribution of each effect.

Our study gives hints about the behavior of hippocampal synapses during burst firing. Facilitation accumulates during a burst, allowing even synapses with low release probabilities to have reliable synaptic transmission when fired repetitively (18). The depressive mechanisms we have described herein, on the other hand, would act to limit the number of releases during a burst. Inactivation in particular may ensure that synapses of high P do not release excessively, because this form of depression may be more pronounced in such synapses (see above). To elucidate the precise effects of these mechanisms on synaptic behavior during *in vivo* burst firing, it will be necessary to determine their quantitative relationship to P and, of course, the effects of temperature on this relationship. In any case, perhaps such mechanisms are used to limit the quantity of transmitter released and thus to conserve the ATP-expensive processes involved in synaptic transmission.

1. Ranck, J. B., Jr. (1973) *Exp. Neurol.* **41,** 462–531.
2. Fox, S. E. & Ranck, J. B., Jr. (1975) *Exp. Neurol.* **49,** 299–313.
3. Kandel, E. R. & Spencer, W. A. (1961) *J. Neurophysiol.* **24,** 243–259.
4. Suzuki, S. S. & Smith, G. K. (1985) *Exp. Neurol.* **89,** 90–95.
5. Larson, J., Wong, D. & Lynch, G. (1986) *Brain Res.* **368,** 347–350.
6. Huerta, P. T. & Lisman, J. E. (1995) *Neuron* **15,** 1053–1063.
7. Otto, T., Eichenbaum, H., Wiener, S. I. & Wible, C. G. (1991) *Hippocampus* **1,** 181–192.
8. Stevens, C. F. & Wang, Y. (1995) *Neuron* **14,** 795–802.
9. Dobrunz, L. E. & Stevens, C. F. (1997) *Neuron* **18,** 995–1008.
10. Raastad, M., Storm, J. F. & Andersen, P. (1992) *Eur. J. Neurosci.* **4,** 113–117.
11. Allen, C. & Stevens, C. F. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10380–10383.
12. Stewart, M., Luo, Y. & Fox, S. E. (1992) *Brain Res.* **591,** 122–128.
13. Zucker, R. S. (1989) *Annu. Rev. Neurosci.* **12,** 13–31.
14. Murthy, V. N., Sejnowski, T. J. & Stevens, C. F. (1997) *Neuron* **18,** 599–612.
15. Schikorski, T. & Stevens, C. F. (1997) *J. Neurosci.* **17,** 5858–5867.
16. Fisher, R. E., Gray, R. & Johnston, D. (1990) *J. Neurophysiol.* **64,** 91–104.
17. Mackenzie, P. J., Umemiya, M. & Murphy, T. H. (1996) *Neuron* **16,** 783–795.
18. Lisman, J. E. (1997) *Trends Neurosci.* **20,** 38–43.