Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4363–4367, May 1992
Neurobiology

Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade

(long-term potentiation/hippocampal slice/synaptic plasticity/learning/memory)

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Communicated by Leon N Cooper, January 28, 1992 (received for review December 30, 1991)

ABSTRACT **We tested a theoretical prediction that patterns of excitatory input activity that consistently fail to activate target neurons sufficiently to induce synaptic potentiation will instead cause a specific synaptic depression. To realize this situation experimentally, the Schaffer collateral projection to area CA1 in rat hippocampal slices was stimulated electrically at frequencies ranging from 0.5 to 50 Hz. Nine hundred pulses at 1–3 Hz consistently yielded a depression of the CA1 population excitatory postsynaptic potential that persisted without signs of recovery for >1 hr after cessation of the conditioning stimulation. This long-term depression was specific to the conditioned input, ruling out generalized changes in postsynaptic responsiveness or excitability. Three lines of evidence suggest that this effect is accounted for by a modification of synaptic effectiveness rather than damage to or fatigue of the stimulated inputs. First, the effect was dependent on the stimulation frequency; 900 pulses at 10 Hz caused no lasting change, and at 50 Hz a synaptic potentiation was usually observed. Second, the depressed synapses continued to support long-term potentiation in response to a high-frequency tetanus. Third, the effects of conditioning stimulation could be prevented by application of NMDA receptor antagonists. Thus, our data suggest that synaptic depression can be triggered by prolonged NMDA receptor activation that is below the threshold for inducing synaptic potentiation. We propose that this mechanism is important for the modifications of hippocampal response properties that underlie some forms of learning and memory.**

A cardinal feature of neuronal responses in the central nervous system is stimulus selectivity. Work on a number of preparations, including the developing visual cortex (1), adult somatosensory cortex (2), and hippocampus (3), has shown convincingly that neuronal selectivity can be modified by experience. It is a widely held belief that such modifications are a likely basis for learning and memory in mammals.

Experience-dependent shifts in selectivity require that neurons acquire responsiveness to new stimuli and lose responsiveness to previously effective stimuli. A critical question concerns the mechanisms whereby this is accomplished. According to a theory developed for visual cortex (4–6), stimulus selectivity will evolve if excitatory synaptic inputs are potentiated when they consistently yield postsynaptic responses greater than a critical value (called the modification threshold, θ_m) and are depressed when they consistently yield responses greater than zero (the average "spontaneous" value) but less than θ_m. Because the value of the modification threshold is a function of the average postsynaptic cell activity (5, 6), the selectivity of a neuron can be modified when the probability of different patterns of input activity shifts, as it would when the animal is experiencing a novel environment.

On the basis of work done on long-term potentiation (LTP) in area CA1 of the hippocampus (7), the proposal was made that this theoretical modification threshold θ_m related to the postsynaptic membrane potential at which *N*-methyl-D-aspartate (NMDA) receptors became sufficiently active to trigger increases in synaptic strength (8, 9). It follows from this hypothesis that patterns of input activity that consistently fail to activate NMDA receptors in a manner that is sufficient to trigger LTP should produce a long-term depression (LTD) of synaptic effectiveness. We have tested this theoretical prediction in the Schaffer collateral–CA1 pathway in rat hippocampal slices. These data were first presented at the 1991 Society for Neuroscience meeting in New Orleans (10).

MATERIALS AND METHODS

The experiments described in this paper were performed on transverse slices prepared from the hippocampi of adult male albino rats (body weight ≈ 200 g). Each animal was given an overdose of sodium pentobarbital (≈75 mg/kg, i.p.) and was decapitated soon after the disappearance of any corneal reflexes. The brain was rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing, in mM, NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $MgCl_2$, 1.5; $CaCl_2$, 2.5; $NaHCO_3$, 26; and dextrose, 10. The hippocampus was dissected free and sectioned into 0.4-mm-thick slices by using a vibrating microtome. These slices were collected in ice-cold ACSF and gently transferred to an interface slice chamber. Here, the slices were maintained in an atmosphere of humidified 95% O_2/5% CO_2 and superfused at a rate of 1 ml/min with 35°C ACSF saturated with 95% O_2/5% CO_2.

After at least 1 hr of equilibration in the slice chamber, a concentric bipolar stimulating electrode was placed in the trajectory of the Schaffer collateral fibers projecting to the stratum radiatum of area CA1. In some experiments a second stimulating electrode was placed on the opposite (subicular) side of the recording location to activate a second converging input. The recording pipette was filled with 1 M NaCl and was placed in the apical dendritic layer of CA1. Population excitatory postsynaptic potentials (EPSPs) were evoked by using 10- to 30-μA stimuli of 0.2-msec duration. These responses were digitized at 20 kHz and stored on a computer. The initial slope of the population EPSP was extracted as a measure of the magnitude of the response.

At the start of each experiment, a full input–output curve was constructed. A stimulation intensity was selected for baseline measurements that yielded between ½ and ⅔ of the maximal response. In general, slices were studied only if the

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Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; EPSP, excitatory postsynaptic potential; LFS, low-frequency stimulation; AP5, DL-2-amino-5-phosphonovaleric acid.

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maximal response amplitude was ≥2 mV and, as a rule, slices were studied for no longer than 6 hr *in vitro*. Baseline measurements were collected by using single shocks every 30 sec. The conditioning stimulation consisted of 900 pulses delivered at frequencies ranging from 1 to 50 Hz. In every experiment an *F* test was used to confirm that any change in the response after conditioning could not be explained by a linearly drifting baseline (11). The summary graphs in Figs. 1–3 were generated as follows: (*i*) the EPSP slope data for each experiment were expressed as percentages of the preconditioning baseline average; (*ii*) the time scale in each experiment was converted to time from the onset of conditioning; and (*iii*) the time-matched normalized data were averaged across experiments and expressed as the means ± SEM.

RESULTS

In an effort to provide a high level of presynaptic input activity without producing a postsynaptic response so large that it yielded LTP, we tried extended periods of low-frequency stimulation (LFS) in the range of 0.5–5 Hz. The lasting consequences of 900 pulses delivered at 1 Hz are illustrated in Fig. 1. The response was often facilitated immediately after the onset of conditioning stimulation, but this was always followed by a progressive decline in the response magnitude during conditioning to values below the initial baseline (data not shown). When the baseline measurements were resumed (at 0.03 Hz), the first response was always depressed, even below the value attained during the LFS (Fig. 1*A*). Although there was usually some recovery in response magnitude over the next several minutes, the population EPSP slope always reached a plateau at a value that was significantly depressed as compared with the pre-LFS control period (Fig. 1*B*).

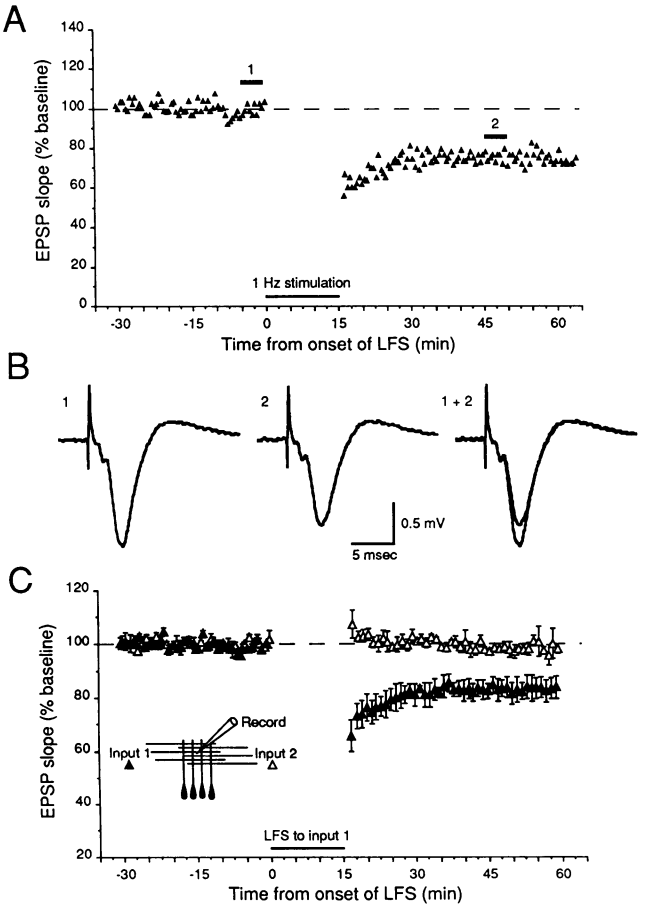

FIG. 1. (*A*) Record of a representative experiment in which LTD was induced by LFS. Each point represents a single measure of the initial slope of the population EPSP evoked by stimulation of the Schaffer collaterals at 0.03 Hz. The horizontal bar represents the period of 1-Hz conditioning stimulation. (*B*) Means of 10 consecutive sweeps before and after LFS conditioning at the times indicated by the numbers in *A*. (*C*) Normalized means (±SEM) of five experiments in which the response to two independent inputs was monitored (*Inset* shows the stimulating/recording configuration). LFS (1-Hz) conditioning stimulation given to input 1 produced a depression only of the response to input 1 (▲).

There are several possible explanations for these results that do not involve plasticity of the stimulated synapses. For example, it is possible that the prolonged LFS produces excitotoxic damage in the target neurons or some generalized loss of postsynaptic excitability. If this were the case, then LFS of one input to a postsynaptic neuron would be expected to produce LTD of a second input that did not undergo conditioning stimulation. However, we found that 1-Hz LFS applied to the Schaffer collaterals had no lasting effect on the response to a second, independent, input (Fig. 1*C*; $n = 5$). As it is likely that at least some fraction of the fibers in the two paths converged onto the same postsynaptic cells, these data can be taken as evidence that the LTD is "input specific" and thus confined to the stimulated synapses.

Input specificity of LTD does not rule out the possibility of permanent damage or neurotransmitter depletion of the conditioned pathway. We attempted to address this concern in several ways. First, we confirmed that the depressed input could still undergo LTP after a patterned high-frequency tetanus (data not shown). This observation does not rule out presynaptic damage or depletion, but it does mean that the depressed synapses are sufficiently viable to support the mechanisms that give rise to LTP. A second approach was to investigate the effect of varying the stimulation frequency. We reasoned that if the LTD was simply explained by the damage caused by prolonged stimulation, then the effect should be relatively independent of stimulation frequency. However, to the contrary, the lasting effects of conditioning stimulation showed a marked frequency dependence. As illustrated in Fig. 2, 900 pulses at 3 Hz resulted in LTD (81% ± 2% of control at 30 min after conditioning; $n = 5$); however, the same number of pulses at 10 Hz produced (on average) no lasting change (101% ± 4% of control; $n = 6$), and the pulses at 50 Hz produced a variable potentiation (121% ± 7% of control; $n = 5$).

The possibility remains that the frequency dependence of the effect is explained by the superimposition of LTP onto the presynaptic damage caused by the conditioning stimulation. However, this explanation is ruled out by a third line of evidence. In six experiments, 200 μM DL-2-amino-5-phosphonovaleric acid (AP5) was added to the bath and then 900 pulses were delivered at 1 Hz. This drug treatment markedly inhibited the induction of LTD (Fig. 3). However, in every case LTD was induced by the same stimulation immediately following wash-out of the AP5. AP5 is known to block postsynaptic NMDA receptors (7) and is not thought to have any significant presynaptic actions. Preliminary work indicates that MK801, an NMDA channel blocker, will also interfere with induction of LTD. Therefore, these data argue strongly that the LTD we report here is a real manifestation of synaptic plasticity in the conditioned pathway, and they suggest a possible involvement of postsynaptic NMDA receptors in the induction mechanism.

DISCUSSION

The major finding of this study is that repetitive stimulation of the Schaffer collateral input to CA1 at frequencies below the threshold for inducing LTP can lead to a reliable and long-lasting synaptic depression. This LTD is specific for the

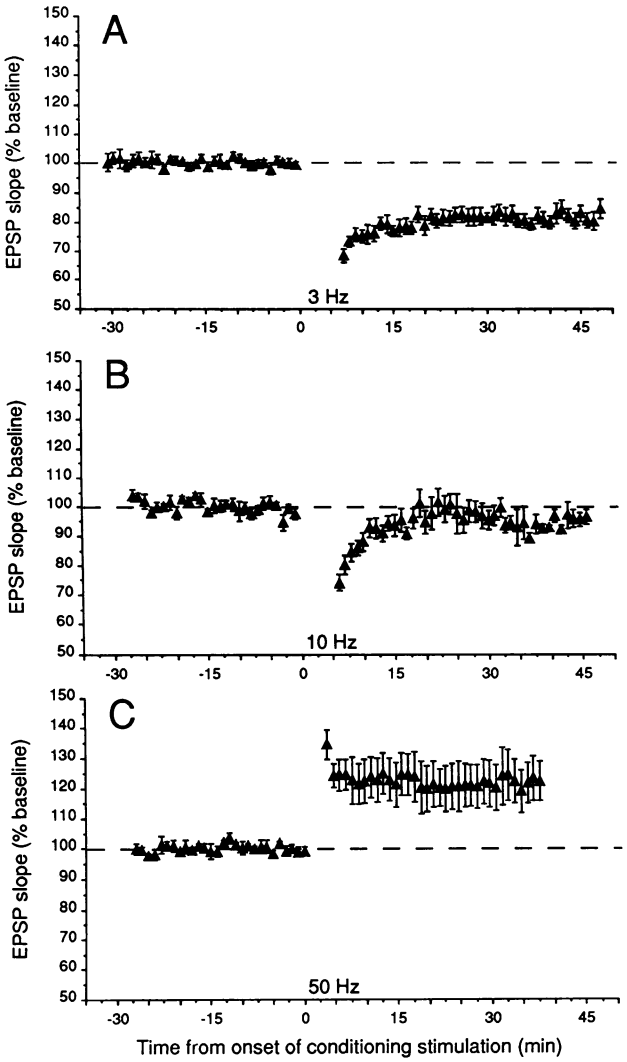

FIG. 2. Normalized averages of experiments in which 900 pulses were delivered at different frequencies. (*A*) 3 Hz, $n = 5$. (*B*) 10 Hz, $n = 6$. (*C*) 50 Hz, $n = 5$. Conventions are as for Fig. 1.

stimulated input and may be triggered, at least in part, by activation of NMDA receptors.

Most of the previous accounts of activity-dependent decreases in synaptic effectiveness in hippocampus have concerned a phenomenon usually called "heterosynaptic" depression (see ref. 12 for review). This refers to a generalized decrease in the response to synaptic inputs that are silent during episodes of strong postsynaptic activation (13–15). Even the postsynaptic activity that accompanies low-frequency (1–15 Hz) antidromic stimulation of CA1 neurons can produce this effect (16). Therefore, it is of significance that the LTD observed in the present study was input specific (Fig. 1*C*). This makes it unlikely that the LTD reported here is simply the manifestation of an orthodromically induced, but nonetheless heterosynaptic, change in synaptic effectiveness.

Depression of synaptic transmission after prolonged periods of repetitive stimulation is not a new observation. For example, at the neuromuscular junction, del Castillo and Katz (17) showed that 2-Hz stimulation causes a progressive decline in the quantal release of acetylcholine. This "post-tetanic depression" is often ascribed to depletion of neurotransmitter, and it has been observed and analyzed at central synapses (cf. ref. 18) recently, including those in CA1 (19). Although this effect is of considerably shorter duration than the LTD we report here, the question nonetheless arises of whether our results can be explained by a lasting depletion of transmitter at the stimulated synapses. In addressing this issue it is instructive to consider that, at the neuromuscular junction, post-tetanic depression is unaffected by postsynaptic acetylcholine receptor antagonists (17). In marked contrast, however, we have found that NMDA receptor antagonists can prevent the induction of LTD in CA1. Because the release of neurotransmitter at the Schaffer collateral–CA1 synapse is not directly affected by these drugs (7), this observation suggests that depletion of neurotransmitter is not a likely explanation for LTD. The blockade of LTD by NMDA receptor antagonists also distinguishes the present work from a previous report of homosynaptic depression in CA1 (20).

As NMDA receptors are thought to be exclusively postsynaptic in CA1, our data suggest that LTD is triggered by a postsynaptic event. Although the nature of this event remains to be determined, it seems likely that postsynaptic Ca^{2+} entry is involved (28). In this light it is interesting to note that Lisman (21) has presented a model that shows the feasibility of using quantitative differences in the activity-dependent changes in postsynaptic Ca^{2+} to determine whether synaptic weights will increase or decrease. Another possibility is that metabotropic glutamate receptor activation in conjunction with an elevation in postsynaptic Ca^{2+} serves as a trigger for LTD (22, 29).

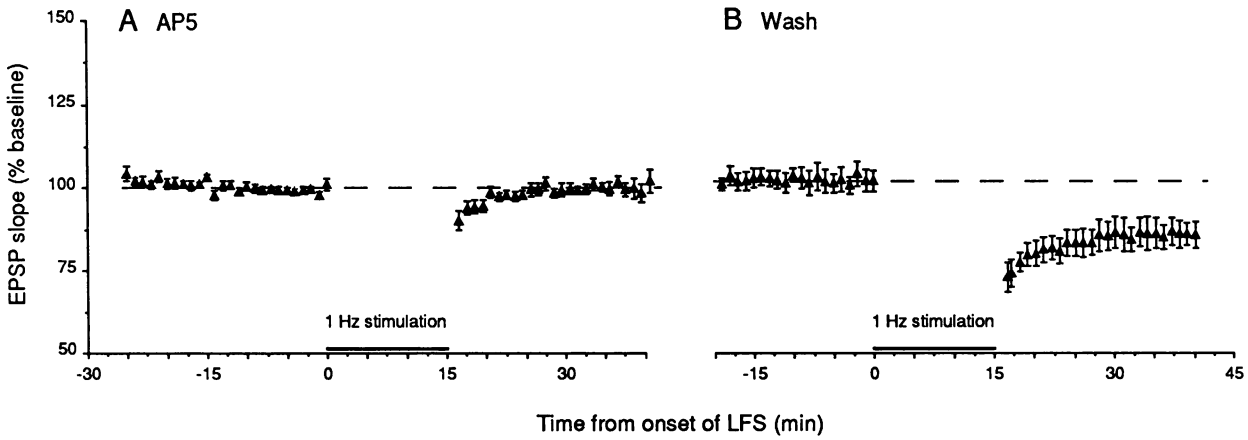

FIG. 3. Effects of an NMDA receptor antagonist on the lasting effect of 900 pulses at 1 Hz. (*A*) AP5 was used in the concentration of 200 μM to overcome any competition for the NMDA receptors that might result from the surge of endogenous transmitter released during stimulation. The EPSP slope was unaffected when the AP5 was introduced, suggesting that the drug at this concentration was still selective for NMDA receptors. (*B*) Removal of antagonist: 30–60 min elapsed between the last point in *A* and the first point in *B* to allow time for wash-out of the drug. All data are expressed as percentage of the baseline prior to the first LFS in AP5, $n = 6$. Conventions are as for Fig. 1.

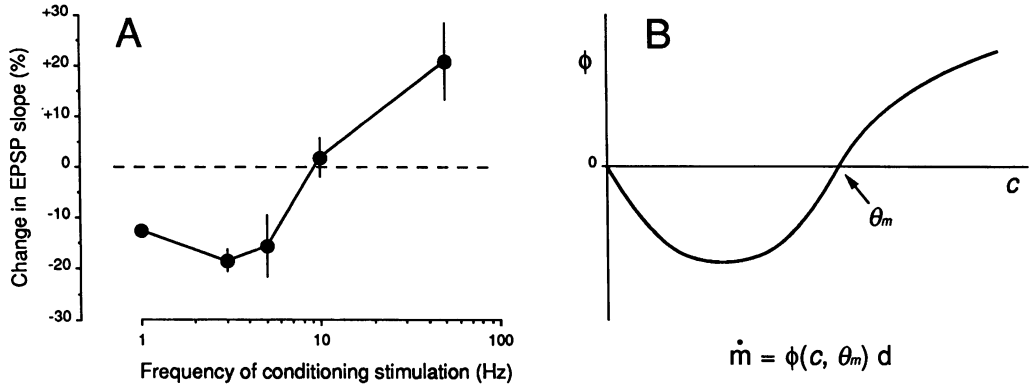

FIG. 4. Comparison of theory and experiment. (*A*) Mean (±SEM) effect of 900 pulses of conditioning stimulation delivered at various frequencies on the response measured 30 min after conditioning; $n \geq 5$ for each point. (*B*) Modification function ϕ of Bienenstock *et al.* (5). Please refer to the text for definition of terms.

Although the LTD documented here is of relatively small magnitude and requires prolonged repetitive stimulation at low frequencies, we believe that it is of physiological significance. The magnitude of the depression can be increased by increasing the number of pulses; for example, we observe LTD of >30% with 1800 pulses at 1 Hz. And, although this may appear to be an extreme type of stimulation, we note that hippocampal electroencephalogram (EEG) recordings in awake rats are characterized by 1- to 10-Hz oscillations that reflect the synchronous firing of hippocampal neurons. Particularly interesting in light of the frequency dependence of the present results (Fig. 2) is the reported shift in the peak of the EEG power spectrum from 1 to 7 Hz as rats go from a state of stillness to active exploration of their environment (23).

Finally, the results of this study may be compared with the theoretical form of modification that inspired it (Fig. 4). According to the theory of Bienenstock *et al.* (5), the modification of excitatory synaptic weight (m) is assumed to proceed as the product of the input activity (d) and a function ϕ of the postsynaptic response (c). For situations where d is a positive constant, then the curve in Fig. 4*B* can be interpreted as the sign and magnitude of the synaptic weight change at different levels of postsynaptic response. If the effects of varying stimulation frequency in the present study are explained by different values of postsynaptic response (perhaps the integrated postsynaptic depolarization or Ca^{2+} level) during the conditioning stimulation, then it can be seen from Fig. 4*A* that our data are in striking agreement with assumptions of this theory.

Another central feature of the theory is the sliding modification threshold (5, 6). That is, the value of θ_m in Fig. 4*B* is assumed to vary as a function of the average postsynaptic response. In this context it is interesting to compare the present results with previous investigations of the effects of LFS in hippocampus. Although earlier reports (24–26) failed to show (or overlooked) LTD in the "naive" hippocampus, they did demonstrate that 1-Hz stimulation applied within several hours of inducing LTP would cause a substantial reduction in the potentiated response. Because this effect is also AP5 sensitive (26), it is possible that "depotentiation" and LTD share a common mechanism, and differ only by degree. This then suggests that the effectiveness of LFS in producing LTD may be dependent on the recent history of activation, or strength, of the synapse. Indeed, although we observe a clear LTD with 1-Hz stimulation in the naive hippocampal slice, we also find that the magnitude of the effect is greatly enhanced after induction of LTP (10). Thus, these data appear to suggest that the other critical assumption of the theory—that of a sliding modification threshold—also may have a plausible physiological basis in the hippocampus (see also ref. 27).

We thank Joel Gold for his assistance in the early phases of this study and Dr. Leon Cooper for many helpful discussions. We also thank the reviewers, whose suggestions improved this paper. This work was supported by an Office of Naval Research Young Investigator Award to M.F.B.

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