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Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation

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Short, high frequency stimulation bursts (4 pulses at 100 Hz) were applied to Schaffer/commissural projections to the CA1 field of rat hippocampal slices at 0.1, 0.2, 1.0 or 2.0-s intervals to assess their efficacy in eliciting long-term potentiation (LTP). Bursts repeated at 2-s intervals induced very little LTP; shorter repetition intervals reliably elicited LTP, with the 200-ms repetition interval producing the greatest potentiation. A short-term potentiation effect, which was maximal 20 s after the last burst and decayed within 10 min, was affected differently by the stimulation parameters than was LTP, suggesting that the two phenomena are due to different processes. The results indicate that patterns of stimulation resembling spike discharge patterns of hippocampal neurons in animals in exploratory situations are effective in inducing LTP and suggest temporal constraints on the mechanisms involved in triggering synaptic plasticity.

There has been much speculation regarding the possibility that the mechanisms underlying hippocampal long-term potentiation (LTP) are involved in memory storage[11]. LTP is usually induced by stimulating afferent pathways with high frequency trains that are considerably longer than the short bursts of action potentials generated by hippocampal neurons in behaving animals. The hippocampal EEG in rats in learning or exploratory situations is typically dominated by slow waves in the 4 to 7 Hz (theta) frequency range (see ref. 7 for a review); single neurons fire short bursts of spikes in phase with this rhythm[1]. It is of interest, therefore, to determine whether electrical stimulation of axons mimicking this pattern can induce LTP. The present experiments were directed at this question.

Hippocampal slices were prepared from male Sprague–Dawley rats (180–220 g) and maintained in vitro in a bath containing (in mM): NaCl 124, KCl 5, KH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 3.4, $MgSO_4$ 2.5, D-glucose 10, L-ascorbic acid 2. Extracellular recording electrodes (1-5 MΩ, filled with 2 M NaCl) were placed in the stratum radiatum of field CA1b to record the dendritic population excitatory postsynaptic potentials (EPSPs) evoked by bipolar stimulating electrodes (twisted 64 μm insulated nichrome wires) placed in the stratum radiatum of field CA1c. Stimulus intensity (biphasic pulses of 0.1 ms duration each half cycle, 40–100 μA) was set to evoke a population EPSP of approximately 2 mV in amplitude ($\bar{X} \pm$ S.E.M.: 1.95 ± 0.02, n = 71).

Each experiment consisted of recording the population EPSPs evoked every 20 s for 10 min before and 20 min after an episode of patterned burst stimulation. The initial slope and peak amplitude of the dendritic response were measured on-line using a MINC-23 minicomputer (Digital Equipment Corp.) and stored on disk. If responses showed a linear drift of less than 10% during the 10-min baseline period (slices in which responses drifted more than this were rejected from analysis), a linear regression was performed on the baseline measures and extrapolated to

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the posttreatment responses to obtain a more accurate assessment of the effects of burst stimulation. This procedure was used in 25 of the 71 experiments reported here; slices on which the correction was performed were randomly distributed in the different stimulation paradigms used (see below).

Patterned stimulation consisted of bursts (4 pulses at 100 Hz, each pulse at twice the test pulse duration) repeated at 2.0, 1.0, 0.2 or 0.1-s intervals (i.e. burst frequencies of 0.5, 1.0, 5.0 or 10.0 Hz, respectively). Each slice was given a single series of 5, 10 or 20 bursts at one of these frequencies. The burst frequency presented to a slice was determined randomly, subject to the constraint that the first 4 slices from a single rat were presented a different frequency. The number of slices used from a single rat ranged from two to six; data were collected from 71 slices obtained from 19 rats.

The results are summarized in Fig. 1 which shows the percentage of cases exhibiting LTP (i.e. a stable increase of at least 10%) and the degree of potentiation of the slope of the population EPSP 20 min after patterned stimulation at different inter-burst intervals and different numbers of bursts. It is evident that bursts repeated at 2-s intervals rarely induced LTP; responses in only 3 of 16 slices showed a stable increase of at least 10%. Bursts presented at 1 Hz were considerably more effective; over 75% of slices stimulated with bursts at 1-s intervals showed stable LTP. A still higher percentage of slices stimulated with bursts every 200 ms (5 Hz) showed stable LTP; moreover, the amount of LTP observed was significantly greater than that obtained after 1- or 2-s interburst intervals. Surprisingly, bursts repeated every 100 ms (10 Hz) were less likely to induce LTP and the amount of potentiation resulting from 5 or 10 bursts at this frequency was significantly lower than that obtained after the same number of bursts presented at 5 Hz. These data indicate that short bursts of axonal stimulation, which do not induce LTP when repeated at long intervals, are effective when repeated more rapidly and that the optimal repetition frequency is in the theta range.

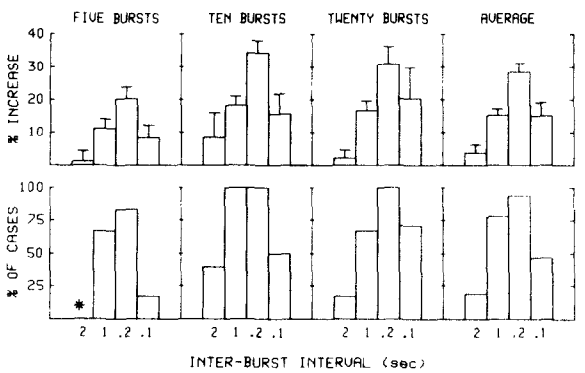

Fig. 1. Magnitude and probability of occurrence of LTP after 5, 10 or 20 bursts at different inter-burst intervals. The top panel shows the average percent increase ($\bar{X} \pm$ S.E.M.; n = 5–7 slices per group) in the slope of the population EPSP 20 min after patterned stimulation. The right-hand panel shows the average LTP across different numbers of bursts. Statistical analysis (t-tests, with significance level of $P < 0.05$) showed that 5, 10 or 20 bursts repeated at 0.2-s intervals induced greater LTP than the same number of bursts repeated at any other interval (except 5 bursts at 1-s intervals and 20 bursts at 0.1-s intervals). LTP was significantly greater after 5 or 20 bursts repeated at 1-s intervals than after the same number of bursts repeated at 2-s intervals. Averaging across all numbers of bursts (n = 16–19 per group), 0.2-s intervals induced significantly greater LTP than all other intervals, 0.1-s intervals were significantly more effective that 2-s intervals, and 1-s intervals induced significantly greater LTP than 2-s intervals. The bottom panel shows the percent of slices in each group showing LTP (defined as an increase of at least 10% which was stable between 10 and 20 min after patterned stimulation). Asterisk marks the fact that no slice in that group showed stable LTP.

Maximal LTP appeared to be induced by 10 bursts at each of the burst frequencies tested. Accordingly, we conclude that the LTP effect saturates after about two seconds of stimulation at the theta frequency.

An episode of patterned burst stimulation typically induced a short-term response facilitation which declined within 10 min to a level of potentiation which remained stable thereafter (Fig. 2). We have followed responses in several slices for several hours af-

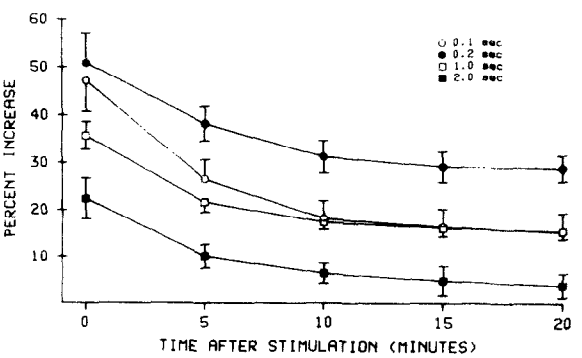

Fig. 2. Time course of LTP after bursts repeated at 0.1, 0.2, 1.0 and 2.0-s intervals. Each curve shows the average potentiation ($\bar{X} \pm$ S.E.M.; n = 16–19 slices per group) of the population EPSP across different numbers of bursts at designated time points after patterned stimulation. The first measurement was taken 20 s after the last burst.

TABLE I

Short-term potentiation after different inter-burst intervals and different numbers of bursts

Upper part shows percent increase in the slope of the population EPSP 20 s after the last burst ($\bar{X} \pm$ S.E.M.). Lower part shows percent increase 20 s after the last burst with percent increase 20 min later subtracted ($\bar{X} \pm$ S.E.M.).

No. of bursts	*Inter-burst interval (s)*			
	2.0	*1.0*	*0.2*	*0.1*
5	13 ± 5	32 ± 4	53 ± 14	38 ± 8
10	37 ± 8	44 ± 7	60 ± 10	62 ± 12
20	19 ± 8	32 ± 3	40 ± 8	42 ± 11
5	12 ± 6	21 ± 6	33 ± 10	30 ± 5
10	28 ± 4	25 ± 9	26 ± 7	46 ± 7
20	17 ± 7	15 ± 5	9 ± 6	21 ± 8

ter 5 Hz stimulation patterns and found the LTP to remain stable after the short-term effect decayed (i.e. after 10 min). In the groups exhibiting significant LTP (at 20 min), the magnitude of the short term effect 20 s after the episode was highly correlated with the degree of potentiation at 20 min (data not shown). Thus, it is possible that the short-term effect represents a transient event or events that serve to trigger the LTP effect. However, as shown in Fig. 2, the degree of response facilitation measured 20 s after 5 or 10 Hz stimulation patterns was virtually identical, although the potentiation remaining 20 min later was considerably larger in the 5 Hz groups. Comparison of the response facilitation 20 s after 5, 10 or 20 bursts at each frequency is shown in Table I. The data in the table make the additional and interesting point that the 20-s facilitation was considerably smaller in the 20 bursts' groups than the 10 bursts' groups for each of the 4 inter-burst intervals, an effect not observed for LTP (see Fig. 1). It appears then that the short- and long-term potentiations respond differentially to frequency of burst presentation and the number of bursts used, an observation that further supports the idea that they are due to different processes[6]. The correlation found between the two in some of the groups may simply indicate that the LTP effect is present as early as 20 s after the bursts and that another, transient form of potentiation rides upon it. If this were so, then an accurate description of the short-term effect could only be obtained by subtracting the stable component (i.e. potentiation at 20 min) from the responses 20 s after burst stimulation; the results of this manipulation are shown on the bottom half of Table I. These data reinforce the suggestion of Racine et al.[8,9] that multiple forms of potentiation may follow high frequency stimulation and also raise the possibility that these are differentially sensitive to stimulation parameters.

The results described here demonstrate that axonal stimulation at frequencies and patterns comparable to those exhibited by hippocampal neurons in behaving animals will produce hippocampal LTP, thereby increasing the likelihood that the potentiation effect is involved in memorial phenomena. Although the behavioral significance of the hippocampal theta rhythm remains unclear, the possibility that it is associated with information-gathering behaviors has been raised[3]; the present results suggest that neuronal activity at this frequency is optimal for the induction of synaptic plasticity in the hippocampus. It remains the case that synchronous activation of large groups of contiguous axons is unlikely to occur in situ; nonetheless, we have recently found that stimulation of the lateral olfactory tract, using patterns similar to those inducing maximal LTP as described here, causes rats to behave as though an odor were present[10]. These observations suggest that stimulation of fiber tracts in certain temporal patterns can produce behavioral effects similar to those produced by natural environmental stimuli. Thus, there is no necessary reason to assume that electrical stimulation produces conditions that differ radically from those obtained when widely spaced cells with convergent projections are simultaneously active.

The results of the present study raise, in a new context, questions about the means through which repetitive stimulation leads to persistent changes in synaptic function. Studies from several laboratories suggest that events in the postsynaptic cell are involved in triggering the LTP effect[2,5,12]. The present results suggest that temporal summation of these effects must occur within a restricted time window (<2 s), since bursts repeated at 2-s intervals produce very little LTP. Two possibilities regarding the nature of these events may be offered: first, that the events which accumulate are directly involved in inducing synaptic modification; second, that the accumulating events 'prime' the postsynaptic cell for synaptic change induced by subsequent synaptic activity. We

recently described experiments in which burst stimulation was presented to two separate sets of inputs to hippocampal neurons, each at 2-s intervals but 200 ms out of phase[4]. Under these conditions, LTP was observed only in responses subsequently evoked by the afferents stimulated 200 ms after a burst in the other set. Thus, it appears that a burst of activity in one set of synapses which does not potentiate those synapses can influence the probability of synaptic change induced by subsequent activity in other synapses on the same neuron. This finding strongly suggests that the short trains of action potentials generated by hippocampal cells in exploring rats serve not only for communication between neurons but also initiate changes that allow subsequent inputs to the same neurons to modify their connections.

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