Blockade of Hippocampal Long-Term Potentiation by Sustained Tetanic Stimulation Near the Recording Site

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INTRODUCTION

Patterned stimulation of excitatory afferent pathways in the hippocampus produces long-term synaptic plasticity widely thought to underlie information storage. In general, low-frequency stimulation (0.5–5 Hz) leads to long-term depression (LTD), whereas high-frequency stimulation (20–200 Hz) elicits long-term potentiation (LTP). However, several investigators reported that high-frequency stimulation, when delivered repetitively or at high stimulus strengths, prevents normal LTP induction; the same stimulation from a second electrode placed farther away subsequently produced LTP at the same recording site. Strong stimulation near the recording site could also interfere with LTP triggered from a distal site. In contrast to sustained high-frequency stimulation, intermittent stimulation (θ burst pattern) delivered close to the recording site produced normal LTP. These data support the hypothesis that strong stimulation releases a factor that acts locally to prevent LTP.

METHODS

Hippocampal slice preparation

Slices were prepared from male Sprague-Dawley rats aged 17–28 days as previously described (McMahon and Kauer 1997). With the use of a vibratome, coronal slices (400 μm) were cut from the middle third of the hippocampus into ice-cold artificial cerebrospinal fluid (ACSF) (in mM: 119 NaCl, 26 NaHCO3, 2.5 KCl, 1.0 NaH2PO4, 2.5 CaCl2, 1.3 MgCl2, 11 D-glucose) saturated with 95% O2-5% CO2. Slices were held after cutting for 1–6 h in an interface chamber at room temperature and then transferred to a recording chamber where the slice was held submerged. The recording solution included the γ-aminobutyric acid-A (GABA-A) receptor antagonist picrotoxin (100 μM), and Ca2+ and Mg2+ were each increased to 4 mM to prevent epileptiform bursting. The bath temperature was maintained between 28 and 30°C.

Extracellular stimulation and recording

Field potentials were recorded from area CA1 with glass microelectrodes filled with 2 M NaCl. Bipolar stainless steel electrodes were generally used to stimulate (Frederick Haer), but in some experiments glass pipettes filled with ACSF and connected via silver wire were used (see RESULTS). Test stimuli were 100 μs in duration, delivered every 10 s. In most experiments, alternating stimulation was delivered to each of two stimulating electrodes, placed in stratum (s.) radiatum so as to activate nonoverlapping groups of afferent fibers. The recording electrode was placed in s. radiatum either “near” (≤200 μm) or “far” (>500 μm) from the stimulating electrode.

LTP inducing protocol

Sustained tetanic stimulation was delivered at 100 Hz for 1 s at 1.5 times the test intensity; this train was repeated twice. In some experiments, a θ burst protocol was also used, consisting of a burst of 4 pulses at 100 Hz, delivered 10 times 200 ms apart; 4 such trains were delivered at 20-s intervals. The stimulus intensity was not increased during θ burst stimulation except where noted. In all experiments, the presynaptic fiber volley was carefully monitored to ensure no change after tetanus; in rare instances in which the fiber volley changed, data were discarded.

RESULTS

Stimulating electrodes were placed on either side of an extracellular recording electrode to activate independent...
groups of Schaffer collaterals. One stimulating electrode was placed \( \approx 200 \, \mu m \) of the recording electrode (near), and a second stimulating electrode was placed \( \approx 500 \, \mu m \) away (far) (Fig. 1A). When a sustained tetanus (100 Hz for 1 s, repeated twice) was delivered through the stimulating electrode near the recording site, LTP was often attenuated or entirely blocked after tetanus (Fig. 1, B and C). However, an identical tetanus delivered 15 min later through the far stimulating electrode produced robust LTP recorded at the same recording site. On average, much less LTP could be generated at the near than at the far stimulation site (LTP at 10 min posttetanus: after far tetanus, 166 ± 11%, \( n = 9 \); after near tetanus, 128 ± 5%, \( n =

FIG. 1. Stimulation close to the recording site attenuates long-term potentiation (LTP); stimulation from a more distant site triggers robust LTP. A: placement of stimulating electrodes, near and far from the recording site. B: example of a recording from a single site with 2 stimulating electrodes. The near pathway (top) receives sustained tetanic stimulation and exhibits no LTP. The same stimulation to the far pathway (bottom) triggers LTP. Inset: field potentials in this and other figures represent the average of 5 responses taken at the times indicated by letters. C: averages of 8 such experiments.
The posttetanic potentiation (PTP) was also smaller after stimulation nearby than after stimulation farther away (PTP #1 min after tetanus: after far tetanus, 212 ± 11%, n = 9; after near tetanus, 152 ± 10%, n = 29). To control for possible nonspecific effects of stainless steel electrodes, additional experiments utilized glass stimulating electrodes. The type of

![Diagram](image-url)

**FIG. 2.** Although a 100-Hz stimulation at 1.5 times test intensity close to the recording site does not elicit LTP, a subsequent θ burst stimulation does. A: placement of electrodes; note 2 recording electrodes. B: example experiment, recording simultaneously both near and far from the stimulus site. Tetanic stimulation triggers little potentiation near the stimulus site (top) but LTP far from the stimulus site (bottom). Subsequent θ burst stimulation produces strong LTP near the stimulus site, with little further potentiation at the farther recording site. Calibration: 0.5 mV, 5 ms. C: average of 5 such experiments.
stimulating electrode made no difference to the result; stimulation near the recording site always produced less LTP than stimulation at a distance (LTP 10 min after near tetanus, with a glass electrode, 126 ± 12%, n = 6).

To investigate further the relationship between LTP and distance, a single stimulating electrode was placed near one recording electrode and far from a second recording electrode (Fig. 2A). A sustained tetanus caused very little LTP at the nearby recording site, whereas robust LTP was simultaneously triggered at the distant recording site (Fig. 2B and C). Moreover, after the sustained tetanus failed to elicit much potentiation at the near recording site, subsequent less intense stimulation (θ burst) effectively triggered LTP. These results emphasize that neither the presynaptic afferents nor the associated synapses are damaged by the strong stimulation, as the relevant synapses can support LTP after weaker stimuli.

Stimulus intensity was routinely increased during the sustained tetani but was not increased during that burst stimulation. To test directly whether the increased current near the recording site alone can account for the lack of LTP, the effects of each stimulus pattern were compared when current intensities were matched (1.5 times test intensity). Sustained tetanus produced 110 ± 4.3% potentiation 10 min after tetanus (n = 5), whereas 10–15 min later θ burst stimulation at 1.5 times test intensity elicited significantly more LTP (141 ± 4.9% potentiation, 10 min after θ burst). These results show that increased current intensity alone does not prevent LTP at a nearby recording site.

Sustained tetanic stimulation might release a diffusible factor that blocks LTP at local synapses (Fig. 3A). This idea was tested by delivering tetanic stimulation simultaneously via two stimulating electrodes, one near the recording site (to release the putative factor) and one farther away (to initiate LTP). LTP, usually triggered by the farther
stimulating electrode, was blocked or attenuated by simultaneous sustained tetanus through the near stimulating electrode (Fig. 3, B and C). Fifteen minutes later, when a sustained tetanus was again delivered only through the far electrode, LTP was triggered. Thus sustained tetanic stimulation interferes with local LTP generation even at synapses not directly activated by the local stimulus.

A previous study showed that multiple θ burst stimulations in area CA1 failed to elicit LTP because of activation of adenosine A<sub>1</sub> receptors (Abraham and Huggett 1997). An A<sub>1</sub> purinergic receptor antagonist, CPT (2–10 μM 8-cyclopentyl-theophylline; RBI), was added in some experiments in which tetanic stimulation was delivered ±200 μm of the recording site. The excitatory postsynaptic potential slope at 5 min post-tetanus was 92 ± 7% of control values, indicating that CPT cannot reinstate LTP in these experiments.

**DISCUSSION**

These experiments support the idea that sustained tetanic stimulation in the hippocampal slice blocks LTP near the stimulating electrode. Tetanic stimulation near the recording site routinely produces less LTP than stimulation far from the recording site. LTP can subsequently be induced with intermittent stimulation of the same synaptic circuit. In addition, the results indicate that a signal may spread heterosynaptically from the locally stimulated site to attenuate LTP at neighboring synapses. Previous work suggested that strong stimulation can block LTP in hippocampal slices (Abraham and Huggett 1997; Bashir and Collingridge 1992). This study demonstrates that one important variable in these experiments is the distance between the stimulating and recording electrodes.

What causes this attenuation of LTP? One possibility is that LTP is induced but masked by a simultaneous synaptic depression (Bashir and Collingridge 1992). This seems unlikely because robust LTP can subsequently be induced by θ burst stimulation, as though the relevant synapses have not already undergone significant LTP. Experiments in which both a near and a far stimulating electrode were simultaneously tetanically stimulated suggest that LTP induction is heterosynaptically blocked by local stimulation. Very strong local depolarization, perhaps because of a large postsynaptic influx of extracellular Ca<sup>2+</sup> or to excessive direct depolarization by the stimulating electrode, could be involved in blocking LTP. In a possibly related finding, heterosynaptic LTD after tetanic stimulation is increased by blocking GABA<sub>A</sub>ergic inhibition, which would increase intracellular depolarization and enhance entry of postsynaptic Ca<sup>2+</sup> (Christie et al. 1994). However, the depression of PTP I observed after nearby strong stimulation suggests a possible involvement of presynaptic terminals.

Anomalies in synaptic transmission were previously noted during recordings close to the stimulus site in hippocampal slices (Dingledine et al. 1987); a stimulating electrode 200 μm from an intracellularly recorded pyramidal cell was less effective in driving the cell to threshold than a more distant stimulating electrode. This result is consistent with the idea that local stimulation effectively limits the excitability of local pyramidal cells.

I hypothesize that strong stimulation causes the local release of a neuromodulator that prevents LTP. Because the blockade of LTP is not maintained over hundreds of microns, the spread of the purported neuromodulator must be limited. It could be released nonspecifically to diffuse a short distance through the local extracellular space or alternatively could be released synaptically from nerve terminals that only travel short distances in the slice preparation. The hippocampus contains afferent fibers that could release myriad neuromodulators during the stimulation used in the current experiments. For example, the axons of local circuit interneurons are known to travel a few hundred microns through s. radiatum and can release a variety of peptides as well as GABA (although GABA<sub>A</sub> receptors are not likely to mediate the lack of LTP because they were blocked in these experiments).

In summary, a mechanism was identified that can locally suppress hippocampal LTP. Although it is not yet known under what physiological conditions this suppressive mechanism is activated, it is clearly seen after high-frequency firing of afferents such as that seen during epileptiform bursting. The results can be explained by the local release of a neuromodulator by strong stimulation. If such a neuromodulator were released in situ, it would be expected to attenuate LTP heterosynaptically near sites of its release.

I thank Drs. Lori McMahon and Susan Jones for helpful comments on the manuscript.

This project was supported by National Institute of Neurological Disorders and Stroke Grant NS-30500.

Received 6 July 1998; accepted in final form 5 October 1998.

**REFERENCES**


