Frequency-Dependent Noradrenergic Modulation of Long-Term Potentiation in the Hippocampus

Abstract. *Norepinephrine, briefly superfused during high-frequency stimulation of the mossy fibers in the rat hippocampal slice in vitro, produced a reversible increase in the magnitude, duration, and probability of induction of long-term synaptic potentiation in the CA3 subfield. Similar results were obtained with isoproterenol, whereas propranolol or timolol reversibly blocked long-term potentiation. Norepinephrine had little apparent effect on responses obtained during low-frequency stimulation of the mossy fibers. These data suggest that norepinephrine can mediate long-lasting, frequency-dependent modulation of synaptic transmission in the mammalian brain. Furthermore, the results suggest a plausible mechanism for some of the known associative interactions between synaptic inputs to hippocampal neurons.*

Long-term potentiation (LTP) of synaptic transmission, as observed in the hippocampal formation, is widely regarded as a candidate substrate for aspects of memory in the central nervous system (*1*). LTP can be induced by brief high-frequency stimulation (*2*); it can last hours or longer (*3*); and, since the concurrent activation of multiple synaptic inputs may be required for LTP, it seems to have associative properties (*4*). Investigation of LTP has focused on the locus and nature of factors critical for its induction (*5*), on its biochemical and ultrastructural correlates (*6*), and on modulatory influences (*7*). The possibility that features of LTP depend on, or are subject to, modulation from neurons whose cell bodies are extrinsic to the hippocampal formation is intriguing.

Recent evidence suggests that the integrity of serotonergic and noradrenergic projections to the dentate gyrus is important for the complete expression of LTP observed at the perforant path–granule cell synapse (*8*). There is evidence of noradrenergic projections to the hippocampus (*9*) and of the ability of norepinephrine (NE) to serve as a neuromodulator (*10*). We now report that brief application of NE during high-frequency stimulation of the mossy fiber pathway reversibly increases the magnitude, duration, and probability of induction of LTP in the CA3 subfield of the rat hippocampal slice in vitro. These effects seem to be mediated by β-adrenergic receptors.

Transverse slices (400 μm thick) from the hippocampi of adult male Sprague-Dawley rats were maintained at 34°C and continuously superfused with an oxygenated Krebs solution (*11*). Standard extracellular recording and stimulating techniques were used.

In the CA3 subfield, there were marked variations among slices both in the intensity of stimulation required to elicit LTP and in LTP duration. LTP was considered to have occurred when input-output curves of population excitatory postsynaptic potential (EPSP) amplitude versus stimulus current were shifted to the left by more than 20 percent 15 minutes after the conditioning train (*12*). LTP duration was quantified as the time at which the amplitude of the population EPSP, with respect to the preconditioning baseline response, had decayed to half the amplitude measured 15 minutes after conditioning (half-decay time). LTP magnitude was measured 15 minutes after the conditioning train. Fifty of the slices from which data were taken (21 percent of the total) showed single or multiple episodes of LTP of the population EPSP amplitude that displayed half-decay times of 20 to 110 minutes (*13*).

Figure 1B illustrates the effect of bath application of 10 μ*M* NE on LTP. The half-decay times for the control and wash LTP episodes were 56 and 25 minutes, respectively, whereas the half-decay time for the episode in which NE was present during high-frequency stimulation was 108 minutes (*14*). In ten experiments, NE produced a three- to fourfold increase in the half-decay time for LTP. In these same ten experiments, NE also produced a reversible increase in LTP magnitude (Table 1).

Qualitatively similar effects on LTP magnitude and duration were observed with the β-adrenergic agonist isoproterenol (1 μ*M*, $n = 4$). The β-adrenergic antagonist propranolol (10 to 100 n*M*) blocked the effect of NE on LTP (Fig. 1C) and caused a reversible blockade of LTP ($n = 5$) without affecting posttetanic

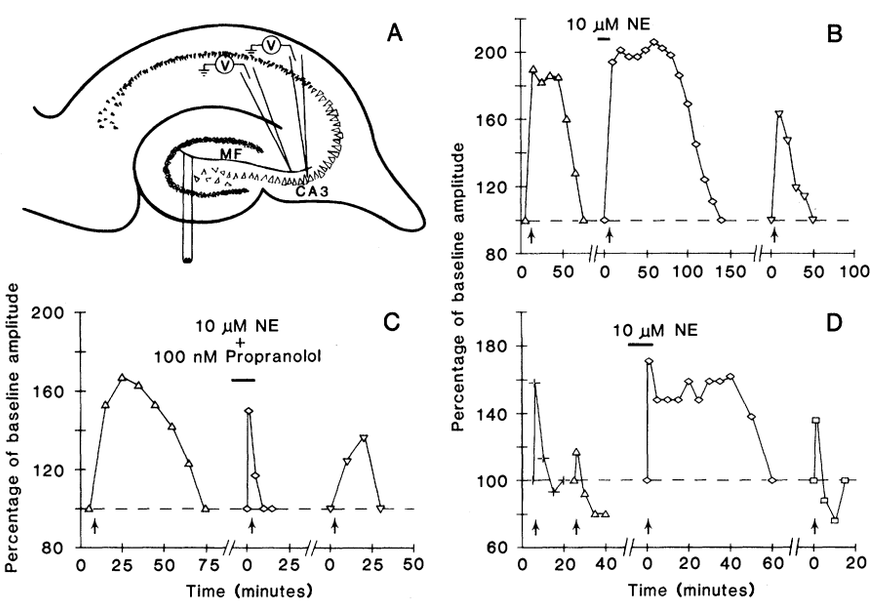

Fig. 1. (A) Schematic diagram of the hippocampal slice preparation, showing the positioning of recording and stimulating electrodes. Abbreviation: MF, mossy fiber. (B) Effect of bath-applied NE (10 μ*M*) on LTP. The arrows indicate the time of the conditioning train (100 Hz, 2 seconds, 50 μA) used to induce LTP. The horizontal bar labeled 10 μ*M* NE depicts the duration of NE application. The symbols represent ten sweep (0.2 Hz) averages of population EPSP amplitudes normalized to the preconditioning average amplitude. The ten stimuli at 0.2 Hz were repeated at 10-minute intervals after conditioning. We chose this procedure for testing response amplitude, which is somewhat different from that normally used, because in preliminary studies we found that the decay rate of LTP was influenced by the frequency of testing. In all figures shown, the standard errors are smaller than the symbols. (C) Effect of 10 μ*M* NE plus 100 n*M* propranolol, a β-receptor antagonist, on the induction of LTP. Data sampled at 1, 5, 10, and 15 minutes after conditioning are shown for the second episode to illustrate PTP. The enhancement of LTP by NE was blocked by propranolol. Propranolol alone also reversibly blocked the induction of LTP without affecting PTP (data not shown). Conditioning parameters were 100 Hz, 2 seconds, 75 μA. (D) Effect of bath-applied NE on the induction of LTP. In the first two episodes, repetitive stimulation (100 Hz, 2 seconds, 30 μA) resulted in brief PTP, followed by a somewhat longer lasting response depression, but no LTP. When NE was present during the conditioning train in the third episode, LTP was induced. Data from episode 4 demonstrate that the effect was reversible.

potentiation (PTP). LTP was also reversibly blocked when propranolol alone was present in the bath during tetanic stimulation ($n = 5$). Although propranolol has well-characterized local anesthetic properties in other preparations (*15*), we obtained similar, reversible blockade of LTP with timolol (100 n*M*) (data not shown), a β-adrenergic antagonist that is much less potent as a membrane stabilizing agent ($n = 10$) (*16*).

LTP is commonly found to be a "threshold" phenomenon; that is, a critical conditioning train intensity seems to be required for its induction (*4*). We questioned whether the addition of NE to the bath during a conditioning train, which had previously not resulted in LTP, would facilitate LTP induction. To test this possibility, we used a procedure beginning with two conditioning trains that resulted in PTP of the population EPSP followed by brief response depression. An identical third train of tetanic stimulation was then applied in the presence of 10 μ*M* NE, and LTP was induced (Fig. 1D) ($n = 4$). This effect was reversible; high-frequency stimulation at the same intensity did not result in LTP after NE had been washed from the bath. Similar results were obtained with 1 μ*M* isoproterenol ($n = 3$).

The drugs used in this study had neither marked nor consistent effects on the input-output curves (obtained at 0.2 Hz) for the population EPSP amplitude versus stimulus intensity taken before the high-frequency conditioning train. At the concentrations used, bath application of NE or isoproterenol resulted in either a small rightward shift in the input-output curve or no change. Since we never observed a leftward shift, these drugs did not augment synaptic efficacy in the absence of high-frequency conditioning stimulation. In five separate experiments, no augmentation of population EPSP amplitude was observed in the presence of NE (10 μ*M*, $n = 3$) or isoproterenol (1 μ*M*, $n = 2$) when the mossy fibers were stimulated at a low frequency (0.2 Hz) for 20 to 30 minutes. Under those conditions, NE and isoproterenol either had no effect on the small response depression commonly observed in control saline, or accentuated the depression. These data, together with the finding that NE or isoproterenol enhances the efficacy of mossy fiber synaptic transmission when accompanied by high-frequency stimulation, suggest that these effects depend on frequency; they are specifically expressed after repetitive stimulation of the mossy fibers.

Recent work in *Aplysia* has shown that the temporal contiguity of the activation of sensory neurons, and of neurons that mediate heterosynaptic facilitation of transmitter release in the sensory neurons, results in greater synaptic facilitation than that produced by heterosynaptic stimulation alone (*17*). Furthermore, this activity-dependent neuromodulation appears to underlie LTP of synaptic connections from sensory to motor neurons in *Aplysia* (*18*). At crayfish neuromuscular junctions, octopamine has been found to mediate activity-dependent increases in synaptic efficacy by a presynaptic mechanism (*19*). Our results are similar in some ways to these forms of activity-dependent modulation, except that we found no enhancement of synaptic transmission by NE in the absence of high-frequency stimulation. We have not yet determined the locus of the effects mediated by NE, nor the temporal requirements on the interaction between tetanic stimulation and NE.

Table 1. Mean (± standard error of the mean) effects of bath application of NE on LTP duration (half-decay time) and magnitude (percentage of baseline) ($n = 10$).

Condition	Duration* (minutes)	Magnitude* (%)
Control	50.9 ± 6.9	149.9 ± 7.5
NE	139.3 ± 13.9†	199.9 ± 15.9†
Wash	34.7 ± 3.7	154.1 ± 8.9

*Significant overall treatment effect: for duration, $F(2, 18) = 20.30$, $P < 0.01$; for magnitude, $F(2, 18) = 8.37$, $P < 0.01$. †Significantly different from control and wash episodes (Scheffé test): for duration, control versus NE, $F = 12.50$, and wash versus NE, $F = 17.50$, d.f. = 2,18, $P < 0.01$; for magnitude, control versus NE, $F = 6.79$, and wash versus NE, $F = 5.70$, d.f. = 2,18, $P < 0.05$.

Our results suggest that activity in NE-containing fibers in CA3 could modulate the consequences of high-frequency mossy-fiber activation if the NE-containing fibers were active at the appropriate time. Our finding that low concentrations of propranolol or timolol can reversibly block the induction of LTP of the population EPSP suggests that tetanic stimulation normally activates surviving NE-containing fibers in the slice and that this heterosynaptic activity modulates the magnitude, duration, and probability of induction of LTP in the CA3 subfield. Some of the known associative properties of LTP might be explained on this basis if it is assumed that the stronger stimulus intensities, which are normally required for LTP and associative LTP, release a suprathreshold amount of NE, which in turn modulates the expression of both LTP and associative LTP (*4*). Our finding of frequency-dependent neuromodulation of the mossy fiber synapses by NE, and the recent voltage-clamp analysis of this synaptic input (*20*), should permit a detailed biophysical study of LTP.

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11. Normal Krebs solution (in millimoles per liter): NaCl, 124; KCl, 3; $CaCl_2$, 2.5; $MgSO_2$, 2; NaH_2PO_4, 1.25; $NaHCO_3$, 26; and glucose 10. This solution was equilibrated with a mixture of 95 percent O_2 and 5 percent CO_2 and constantly perfused at a rate of 1 ml/min throughout the experiment. The *p*H of this solution was 7.4 with or without 20 μ*M* ascorbate.
12. PTP decays within 10 minutes in the CA3 subfield (unpublished observations). Therefore, response enhancement 15 minutes after conditioning was defined as LTP.
13. Half-decay time was chosen to quantify LTP decay because there was usually less uncertainty in its measurement than in total LTP duration. LTP whose magnitude decays within the lifetime of a slice in vitro will be termed decremental LTP. Decremental LTP, in the context of these experiments, has three distinguishing characteristics: (i) The input-output curve at 15 minutes postconditioning is shifted to the left by at least 20 percent. (ii) The response amplitude decays gradually, not precipitously. (iii) More than one episode of decremental LTP with similar half-decay times can usually be elicited from slices that display it. Apparent nondecremental LTP is also commonly observed in the CA3 subfield; in several other experiments, we have found that if the response in normal saline has not started to decay by 60 minutes after conditioning, it will not decay for the lifetime of the slice. Conversely, decremental LTP manifests itself within 60 minutes.
14. The procedure was identical in all experiments in which the effects of drugs on LTP duration and magnitude were assessed. After the control LTP episode, the drug was applied for a standard 20-minute period, after which new input-output and baseline responses were sampled. Baseline responses (5 minutes, 0.2 Hz) were always sampled immediately before a conditioning train for a given episode of LTP. The reasons for this are (i) to assess the stability of the preparation, (ii) to compare the postconditioning response amplitude with responses immediately before the conditioning train, and (iii) to assess

possible drug-induced changes in the responses with 0.2-Hz stimulation before the LTP episode. There was a 10-minute period between the end of the second LTP episode and the conditioning train for the third (wash) episode. During this time, input-output and baseline responses were again obtained. A final series of input-output responses was taken at the end of the experiment.

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Uncertainty of Histologic Classification of Experimental Tumors

Konstantinidis *et al.* (*1*) reported that a single systemic dose of a rapidly metabolized carcinogen promoted the development of malignant tumors at sites of chronic inflammation. In support of this conclusion, they stated that 14 of 47 carcinogen-treated rats developed malignant "soft tissue tumors" at a focus of buccal mucosal irritation, whereas other carcinogen-treated or control rats developed only "hyperplasia and severe inflammatory infiltration." Although the authors implied that some rats died as a result of malignancy [see reference 4 in (*1*)], no actual data were presented regarding the biologic behavior of "malignant tumors" as opposed to benign or hyperplastic lesions. Instead, cellular proliferations apparently were classified as malignant tumors because of their histologic features (legend to table 1).

The use of histologic criteria to assess the "malignancy" of tumefactions in laboratory rodents (*2*) is particularly convenient in large studies of experimental carcinogenesis because it permits animals to be killed as soon as their "tumors" are palpable. The lesions then are classified on the basis of cytologic or histologic features generally associated with malignancy, such as hypercellularity, hyperchromatism, increased mitotic activity, and apparent local invasion. However, the predictive value of these criteria can vary markedly depending on tumor type, organ, and species. As a result, the validity of individual cytologic or histologic indicators of malignancy must be established separately for each histologic variety of neoplasm under investigation (*3*). Spindle cell proliferations, which accounted for 10 of the authors' 14 cases, are among the most difficult to classify by histology. In man, certain spindle cell lesions with extreme cellular pleomorphism and hyperchromatism rarely, if ever, metastasize and are generally cured by simple excision (*4*). Other benign proliferations of fibroblasts characterized by hypercellularity, mitotic activity, and apparent invasion of adjacent connective tissue may not even represent true neoplasms (*5*). In contrast to human spindle cell tumors, which have been studied extensively, spindle cell proliferations in murine rodents are poorly understood (*6*). Although they are among the more common experimentally induced "tumors," they rarely occur spontaneously. Furthermore, the widespread practice of killing experimental animals soon after their lesions have developed has left unanswered many questions about their natural history.

It has been recognized for decades [see, for example, (*7*)] that histologic confirmation of metastatic growth represents the most convincing proof of a tumor's malignancy. Alternatively, certain neoplasms that rarely metastasize are regarded as malignant because they grow relentlessly and invade contiguous normal structures such as blood vessels, muscle, or bone. Tumor size is an unreliable criterion of malignancy since even benign lesions can occasionally achieve great bulk (*8*). Once the natural history and histologic features of a particular class of tumors is well understood, it would seem reasonable, under certain circumstances, to evaluate these lesions by histology alone. This is clearly indicated in the management of human neoplasms because the objective is to intervene before the disease's natural history has become clinically evident. It is less easily justified in work with experimental animals, however, and is particularly unsuitable during investigations of new mechanisms of carcinogenesis.

Konstantinidis *et al.* (*1*) provided no data about tumor metastasis, pattern of local invasion, tumor size, or associated morbidity and mortality. Although, in their report, the authors referred to the lesions listed in table 1 as "histologically malignant," in my opinion the findings illustrated in figure 1 are difficult to distinguish from inflammation accompanied by fibroblast proliferation. Furthermore, all of the cytologic features mentioned in the figure legend may be exhibited by benign neoplasms or hyperplastic processes. The reference to the "dropping-off phenomenon" (legend to figure 1D) and the fact that the authors classified separately the nine "malignant soft tissue tumors" and the single "fibrosarcoma" suggest that they considered the former to be derived from epithelial cells rather than fibroblasts. Making this distinction is particularly difficult (*4*) and ideally should be confirmed by electron microscopy (*9*). Finally, the method used to determine the interval until tumor appearance should be clearly explained, since most rats presumably had preexisting lesions related to hyperplasia and inflammation.

The observations of Konstantinidis *et al.* may have important implications and should be supported by additional information about the actual biologic behavior of the induced lesions. Without such data, the nature of these lesions, and therefore the conclusions of the study, remain unsettled.

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3. In epithelial tumors of the canine perianal gland [H. A. Smith, T. C. Jones, R. D. Hunt, *Veterinary Pathology* (Lea & Febiger, Philadelphia, ed. 4, 1972), p. 233], for example, the only reliable criterion of malignancy is individual cell invasion of adjacent connective tissue. Lesions that appear histologically malignant in other respects but lack invasion pursue a benign course. The canine cutaneous histocytoma (*ibid.*, p. 238) also satisfies many cytologic and histologic criteria generally associated with malignancy; it is hypercellular, hyperchromatic, and has numerous mitoses. It also invades subcutaneous fat. However, these lesions spontaneously regress or are cured by local resection.
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8. It is important to recognize that any large neoplasm near a vital structure can cause morbidity or mortality. For example, a large, noninvasive, histologically benign tumor of the oral cavity or esophagus may result in death by inanition. Thus, lethality is not unequivocal proof of malignancy. Alternatively, certain unusual neoplasms with innocuous cytologic features have a relatively benign clinical course even though they spread by implantation [C. G. Julian and J. D. Woodruff, *Obstet. Gynecol.* **40**, 860 (1972)], vascular invasion, or even metastasis [H. J. Norris and T. Parmley, *Cancer* **36**, 2164 (1975)]. These lesions appear to occupy a border zone between clearly malignant and strictly benign neoplasms.