tions between cones of different spectral types contradicts previous reports (*2–4, 6*). These studies were mainly based on matching linear responses evoked by colored stimuli in the dark-adapted state (*2–4*). Only in one case (figure 4 in *4*), was the effect of a red background on linear responses to red and green stimuli examined. We studied ten red cones in detail using colored backgrounds of different intensities and observed that the effect of the background varied from cell to cell and depended on background intensity. These variations may explain the discrepancy between our and previous reports. The apparent lack of coupling between red and green cones described by others (*6*) suggests that the interaction may be too weak to be measured with simultaneous recordings from two single cones; it can be measured when the summated contributions of many cones impinge upon the test cone, however.

The direct excitatory input between cone photoreceptors of differing spectral types described here adds an additional degree of complexity to information processing at the first synaptic level of the turtle retina. This new pathway must be considered in the retinal mechanisms subserving color vision.

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12. Because excitatory interaction have been demonstrated between red and green members of double cones (*8*), we ensured that impaled "red" cones were not the red members of double cones by measuring their red and green flash sensitivities. The ratio of these sensitivities is consistent with the values measured by others (*2*) for red single cones. Occasionally red members of double cones were impaled; their ratio of red to green sensitivity was approximately 1/8 that of single red cones.
13. Dim test flashes delivered to either the dark- or light-adapted retina evoke photoresponses that are linearly related to the quantal content of the test flash; doubling the quanta in a test flash will double the entire response [(*2, 4*); R. A. Normann and P. J. Anderton, *Vision Res.* **23**, 1731 (1983)]. Accordingly, we tested for response linearity at each background by subtracting eight responses to a dim test flash intensity from four summed responses of twice this intensity. If this operation resulted in no net response (indistinguishable from the baseline noise), all 12 responses were in the cones' linear range.
14. Under each state of adaptation, the set of green test flashes was interleaved between two sets of red test flashes, and the data were rejected if the amplitudes of the two sets of averaged red flash responses differed by more than 10 percent. Further, dark-adapted red and green sensitivities were measured before and after the periods of light adaptation to verify that the cell's sensitivity had not deteriorated over the course of the experiment. This procedure was successfully followed in experiments conducted on ten red cones.
15. Linear sensitivity measurements can be meaningfully applied to a cone only if its excitatory receptive field is fully illuminated by the test spot. For most cones studied, 180 μm (diameter) test spots were large enough to fully illuminate the receptive field of the cone. In some cones, however, enlarging the spot to 320 μm produced a slight increase in the linear response amplitude. In these cones, 320 μm test spots were used.
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Long-Term Potentiation of Hippocampal Synaptic Transmission Affects Rate of Behavioral Learning

Abstract. *Electrical stimulation techniques were used to produce a long-lasting potentiation of synaptic transmission in the hippocampus of naïve rabbits. Animals were then classically conditioned. Long-term potentiation of the hippocampus before training increased the rate at which animals subsequently learned the conditioning task. This result has significance for potential cellular mechanisms of associative learning.*

Identifying cellular mechanisms that are the basis for neural plasticity and its mediation of behavioral learning is a major goal of research in the neurosciences (*1*). Experimental models range from (i) awake, learning animals in which changes in cellular function can be compared directly with changes in behavior to (ii) semi-intact or isolated preparations of the nervous system in which changes in cellular function represent neural analogs of the learning process. Classical conditioning of the rabbit nictitating membrane (NM) response has recently become widely used to study mammalian learning (*2, 3*). In a series of studies, my colleagues and I have found that substantial changes in the activity of hippocampal pyramidal neurons occur during classical conditioning of the NM response and do not occur under other, nonlearning conditions (*4, 5*). This neural plasticity is long-lasting (at least days), robust, and functionally related to the animal's learned behavior (*6*). Foremost among the isolated central nervous system (CNS) preparations is the mammalian hippocampal explant or slice (*7*), which has been used to study long-term potentiation (LTP) (*8*). Such LTP is characterized by a long-lasting (hours to days) increase in synaptic efficacy produced by high-frequency stimulation of afferents to either the hippocampal granule or pyramidal cell populations. The latter effect is also robust and long-lasting and is currently being studied as a model of neural plasticity in the mammalian brain (*9*).

Much debate has centered on whether LTP or an LTP-like phenomenon is the basis for cellular plasticity observed in the intact, behaving animal (*10*). Resolution of this issue would have important implications because several mechanisms already identified as underlying LTP (*11–15*) may be generalizable to associative learning in the intact animal. In previous studies we have noted similarities between the characteristics of hippocampal pyramidal cell plasticity during NM conditioning and the cellular plasticity characteristic of LTP. For example, LTP and the changes in pyramidal cell activity that occur during NM conditioning develop with similar time

courses, change by similar magnitudes from baseline measures, are long-lasting, and are induced with relatively few stimulations (whether peripheral or electrical) and by only specific ranges of stimulation density (*16*). On the basis of these similarities, we suggested that LTP or an LTP-like phenomenon may be the basis for hippocampal neural plasticity during associative learning (*5, 16*). Support for this hypothesis comes from research showing an increase in efficacy of synaptic transmission in the hippocampal perforant path–dentate synapse during NM conditioning (*17*). The results presented here further confirm a link between LTP and associative learning by showing that LTP of the perforant path–dentate synapse in naïve animals results in enhanced rates of learning a two-tone discrimination task as measured by the rabbit NM response.

Male New Zealand White rabbits were anesthetized with halothane and implanted with long-term bipolar stimulation electrodes (*18*) in the left perforant path and a recording electrode in the granule cell layer of the ipsilateral dentate gyrus (Fig. 1A). Precise locations of stimulation and recording electrodes were those that elicited extracellular field potential currents characteristic of monosynaptic activation of dentate granule cells (*19*). After 2 weeks of recovery, animals were randomly assigned to one of two groups: (i) those given high-frequency stimulation of the perforant path to induce LTP and (ii) as a control, those given perforant path stimulation at a low frequency (one every 30 seconds) that does not induce LTP. On the first day after the recovery period, animals were placed in a restrainer in a room separate from the conditioning apparatus, and a series of stimulation voltages (0.1-msec duration, biphasic pulses delivered every 30 seconds) was used to construct baseline input-output functions of perforant path–dentate synaptic efficacy. Depending on their group assignment, animals were then given either high-frequency stimulation (LTP group: eight trains of 400 Hz for 20 msec per train; trains separated by 15 seconds) or further low-frequency stimulation (control group: 64 electrical pulse stimulations delivered at a rate of one every 30 seconds). Animals in both groups received an equal total number of stimulation pulses (*20*). High-frequency stimulation was given at a voltage that produced approximately 75 percent of the maximum spike amplitude. Animals remained restrained for 60 minutes after all stimuli were presented. This procedure was repeated for the next four consecutive days for a total of 5 days.

On day 6, animals were adapted to restraint within the sound-proof conditioning chamber for 90 minutes; no conditioning stimuli or perforant path stimulation was given. On day 7, discrimination training began, with tones used as conditioning stimuli (CS's) and corneal airpuff to the left eye as the unconditioned stimulus (US). Procedures have been described (*4*) and generally conform to those established by Gormezano (*3*). Either a 1-kHz or a 10-kHz tone (850 msec; counterbalanced design) was always paired with the airpuff (100-msec duration). The remaining tone, the CS−, was never paired with the airpuff. The interstimulus interval was 750 msec, with tone and airpuff coterminating. Animals were given 12 blocks of trials per day, with each block consisting of eight CS+ and eight CS− trials. The order of CS+ and CS− trials was a pseudorandom sequence. The intertrial interval was pseudorandomly varied at 20, 30, or 40 seconds (mean, 30 seconds). Animals were conditioned to a criterion of > 80 percent conditioned response (CR) rate to the CS+ and < 20 percent CR rate to the CS− as measured during the last half of the training session. A minimum of 0.5 mm NM movement within the CS-US interval was the criterion of a CR.

High-frequency stimulation of the perforant path produced LTP of synaptic transmission. By 4 days (96 hours) after the first series of high-frequency trains, all animals in the LTP group ($N = 8$) exhibited significantly (at least $P < 0.01$) enhanced amplitudes of the population spike (Fig. 1, B and C). Pre-LTP and post-LTP differences were determined with a Wilcoxon test for matched samples on the entire input-output curve. The same analyses of control animals ($N = 8$) revealed no statistically significant differences in six of eight cases and a significant ($P < 0.05$) depression relative to day 1 values in two cases. Thus, all of the LTP animals and none of the control animals exhibited enhanced perforant path–dentate synaptic transmission.

Behavioral results showed that animals in the LTP group learned the discrimination task significantly faster than control animals. (i) Animals given high-frequency stimulation required significantly fewer ($\bar{x} = 6.24$) trials than control animals ($\bar{x} = 864$ trials) to reach the behavioral conditioning criteria [$t(14) = 3.10$, $P < 0.01$]. (ii) Although animals required various numbers of training tri-

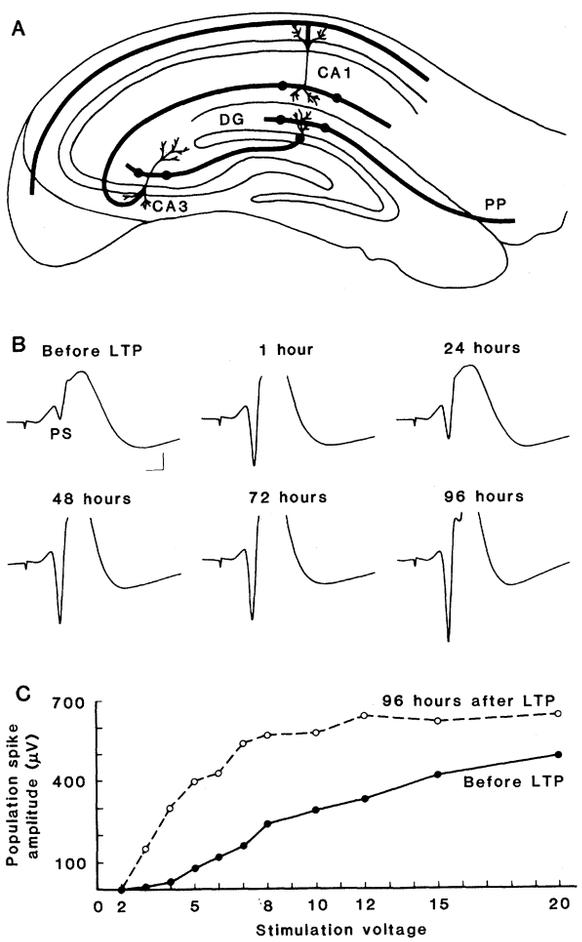

Fig. 1. (A) Schematic diagram of a section of the rabbit hippocampus taken transverse to its longitudinal axis. Stimulating electrodes were implanted in the perforant path (*PP*) and recording electrodes in the granule cell body layer of the dentate gyrus (*DG*). Single shock stimulation of perforant path fibers monosynaptically activates granule cells and produces synaptic and action potential currents recorded as field potentials. (B) Field potentials recorded from the dentate gyrus of one animal over the the 5 days preceding behavioral conditioning, before and after LTP was induced. All potentials shown were induced by a constant voltage (5 V) [see input-output curve in (C)]. High-frequency stimulation enhanced the population spike (*PS*) 1 hour later; the enhancement was still present 24 hours later. Subsequent high-frequency trains produced further enhancement of the population spike at 48, 72, and 96 hours. Calibrations: 100 μV, 2 msec. (C) Input-output curve showing the effect of high-frequency stimulation of the perforant path at all voltages tested for the same animal shown in (B), 96 hours after the initial high-frequency train.

als to reach this behavioral criterion, all animals were conditioned for at least two sessions. Animals in the LTP group exhibited significantly more conditioned responses to the CS+ than control animals on both the first [$t(14) = 4.61$, $P < 0.01$] and the second [$t(14) = 4.43$, $P < 0.01$] days of discrimination conditioning (Fig. 2A). The LTP and control groups did not differ statistically significantly in their response rates to the CS− [$t(14) = 0.004$, day 1; $t(14) = 1.17$, day 2].

To ensure that the behavioral differences between LTP and control groups were not due to the arbitrariness of our criteria, we selected three additional criteria with which to compare animals' performance (Fig. 2B). Animals in the LTP group reached all three of these additional criteria in significantly fewer trials than control animals (*t*-tests).

Long-term potentiation of synaptic transmission in the hippocampus of naïve animals accelerates subsequent classical conditioning. Because differential behavioral conditioning was enhanced, LTP did not generally increase responsivity or sensitivity of the organism. This result has several important implications. First, it suggests that the cellular mechanisms underlying LTP may also be the basis for learning-induced changes in hippocampal unit activity during NM conditioning, and possibly during other types of associative learning as well. Current evidence suggests that LTP may be due to one or a combination of the following: increases in the amount of neurotransmitter released from presynaptic terminals (*11*), changes in postsynaptic dendritic spine morphology (*12*), increases in the number of postsynaptic receptors (*13*), and increased synthesis of specific macromolecules (*14*; also see *15*). Thus, similar mechanisms may be the basis for hippocampal cellular plasticity in intact learning animals as well; a recent report showing that rabbit NM conditioning is accompanied by increases in the number of glutamate binding sites in the hippocampus (*21*) supports this hypothesis.

Second, together with our unit recording (*4, 5*) and lesion studies (*6*), these data demonstrate an important functional role for the hippocampus during classical conditioning of the NM response. During the course of NM conditioning, hippocampal pyramidal neurons gradually increase their frequency of firing and also develop a within-trial pattern of discharge that parallels or models the shape of the conditioned NM response (*4*). Bilateral removal of the hippocampus prevents NM learning in complex tasks such as discrimination reversal and stimulus blocking, and it also disrupts the shape of conditioned NM responses during reversal training (*6, 22*). Experimental manipulations that disrupt the activity of an intact hippocampus also disrupt conditioned NM responding in simpler learning tasks such as a one-tone, delay procedure (*6*). The data reported here are consistent with the experiments on simple learning in demonstrating that enhancing hippocampal synaptic transmission enhances the rate of learning a two-tone discrimination task even though the hippocampus is necessary only for more complex learning tasks (such as two-tone reversal). Long-term potentiation of hippocampal synaptic transmission may enhance the rate of NM learning by enhancing the rate at which the pyramidal cell model of conditioned NM behavior develops during training, by increasing the amplitude of the pyramidal cell model, or both. For example, increasing the efficacy of perforant path–dentate synaptic transmission may result in an increased within-trial excitability of pyramidal neurons because dentate granule cells provide excitatory input to the pyramidal cell population (*23*) and are excited by CS presentation during classical conditioning of the NM response (*17*).

Finally, this experiment demonstrates that the identical procedures used to induce enhancement of synaptic transmission in the isolated hippocampal slice also have behavioral consequences when conducted in the intact animal. Thus, a phenomenon identical or similar to LTP may occur during associative learning in the normal animal.

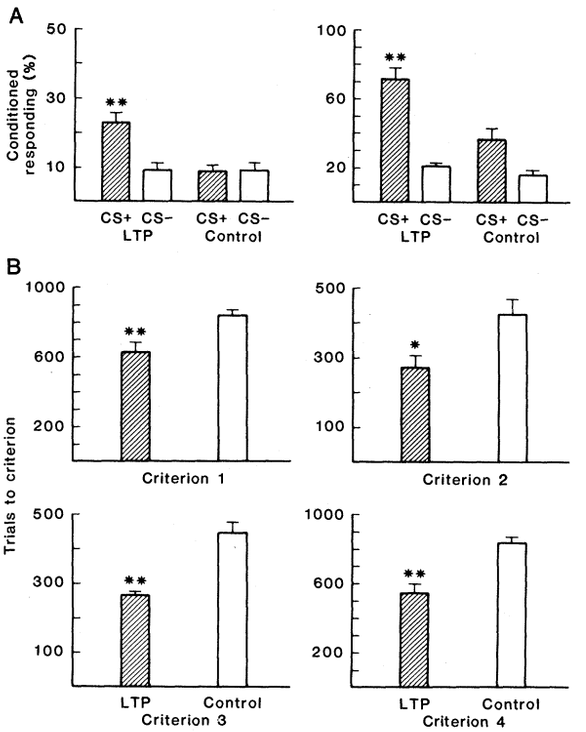

Fig. 2. Effects of LTP (means ± standards errors of the mean) on classical conditioning of the rabbit NM response. (A) Average conditioned responding to the CS+ and CS− on days 1 and 2 of discrimination training for animals given prior high-frequency stimulation of the perforant path (LTP) and for those given only low-frequency stimulation (controls). (B) Average trials required for animals in LTP and control groups to reach criteria of (criterion 1) > 80 percent CR to CS+ and < 20 percent CR to CS−; (criterion 2) five consecutive CR's to CS+; (criterion 3) two consecutive blocks of at least 50 percent CR to the CS+; and (criterion 4) > 80 percent CR to CS+ and at ≥ 60 percent difference in CR rates to CS+ and CS−. $^{**}P < 0.01$. $^{*}P < 0.05$.

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18. Stimulation electrodes were a pair of stainless steel insect pins (00) insulated with Expoxylite. The tips were scraped to expose 200 to 250 μm^2 of uninsulated surface. They were separated by 1 mm in the medial-lateral plane. Recording electrodes were stainless steel insect pins (00) etched to a taper in 10 percent HCl and then insulated with Epoxylite. Tips were scraped to expose approximately 50 μm^2 of uninsulated surface. Impedances typically measured 500 kilohm to 1 megohm when tested at 135 Hz in vitro.
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Multicentric Origin of Colon Carcinoma

Hsu *et al.* (*1*) report that colonic polyps in patients with Gardner syndrome are multiclonal in origin. They pose the question of whether colonic carcinomas arising in such patients would also be multiclonal.

In fact, a colon carcinoma of a black female heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency was found to be multiclonal in origin by E. Beutler *et al.* (*2*). Hsu *et al.* suggest that this observation "may have been due to stromal contamination in the samples." We (*2*) are incorrectly cited as being one of the sources of this suggestion. The actual findings were that some metastases contained primarily G6PD-A and others primarily G6PD-B. Only 7 of 24 tumor nodules contained equal amounts of G6PD-A and G6PD-B, and we proposed that in these nodules the findings might be due to stromal contamination. The fact that many tumors in the patient contained either G6PD-A or G6PD-B was interpreted unequivocally as showing that the origin of the tumor was multicentric. Although these observations are more than 15 years old, we still consider them to be valid. While they may not answer the question specifically posed regarding Gardner syndrome, they do show that colon carcinoma may, at least at times, be multicentric in origin. One can only speculate regarding the mechanism that transforms several cells to give rise to what seems to be a single neoplasm. It could be due, for example, to a viral infection transforming a patch of cells rather than a single cell.

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We agree that our summary of the study by Beutler *et al.* (*1*) was too brief to do justice to that work. Their findings that the primary tumor contained both G6PD isoenzymes may indeed have been due to stromal cell contamination; they pointed out that the primary tumor was mostly fibrous stroma (*1*). We did not include their findings that most of the liver and omental metastases contained either G6PD-A or G6PD-B. Beutler *et al.* did not unequivocally interpret those findings as showing that the origin of the tumor was multicentric. Rather, they stated that "the studies . . . suggest that the metastases represented clones . . . [and] it appears that this tumor arose from . . . a patch of cells. . . ." In the summary, the authors stated that "the findings were regarded as consistent with a multicentric origin of carcinoma of the colon" (*1*).

Furthermore, the finding of metastases containing either G6PD-A or G6PD-B in most cases and both isoenzymes when stromal contamination was present is consistent with a clonally derived primary tumor and the presence of an undetected synchronous or metachronous clonal primary tumor or tumors. Synchronous and metachronous colon cancers occur not infrequently in advanced colon cancers (*2*); and the patient described by Beutler *et al.* had advanced cancer and died 3 months after laparotomy for obstruction (*1*). Therefore, although the study of Beutler *et al.* suggested a multicentric origin, we do not yet have unequivocal documentation of the colonal origin of colon carcinoma.

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