Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9280–9284, December 1990
Neurobiology

Postsynaptic Hebbian and non-Hebbian long-term potentiation of synaptic efficacy in the entorhinal cortex in slices and in the isolated adult guinea pig brain

(synaptic plasticity/intracellular recording/*N*-methyl-D-aspartate/ionic channels)

A. ALONSO*, M. DE CURTIS, AND R. LLINÁS†

Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016

Contributed by R. Llinás, August, 15, 1990

ABSTRACT **Long-term potentiation (LTP) was investigated in the mammalian entorhinal cortex by using two *in vitro* preparations, the isolated brain and the entorhinal cortex slice. Hebbian and non-Hebbian types of LTP appear to be present in layer II entorhinal cortex cells as demonstrated using two protocols: (*i*) tetanic stimulation of the piriform-entorhinal cortex afferent pathway to generate homosynaptic potentiation and (*ii*) postsynaptic subthreshold rhythmic membrane potential manipulation not paired to presynaptic activation, which gives rise to non-Hebbian LTP. The induction and the expression of both types of LTP were found to be dependent on activation of *N*-methyl-D-aspartate receptors as shown by their sensitivity to the receptor agonist D-2-amino-5-phosphonovalerate. This is in contrast to LTP in the hippocampus [Zalutsky, R. A. & Nicoll, R. A. (1990) *Science* 248, 1619–1624], where LTP is expressed by quisqualate receptors. Since, in the entorhinal cortex, LTP is linked to a selective increase of the *N*-methyl-D-aspartate-receptor-mediated synaptic responses, this enhancement is most likely due to postsynaptic factors.**

The study of synaptic plasticity is of great relevance in defining the self-organizing properties of the central nervous system. One such plastic phenomenon is the long-term potentiation (LTP) of synaptic efficacy, which has been intensively investigated in the hippocampus, where it was originally discovered (1). Little is known, however, about the repertoire of synaptic plasticity in other cortical structures. The neurons in layer II of the entorhinal cortex (EC) are of particular interest in this respect as they demonstrate LTP and in addition give rise to the main component of the "perforant path," the major source of extrinsic input to the hippocampus (2).

The study of the LTP of synaptic efficacy and of its possible relation to certain forms of "memory" has been the subject of many studies *in vitro* and *in vivo* (3, 4). Setting aside the issue of whether LTP may relate to some aspects of memory acquisition and/or recall, we describe here a set of unique characteristics of LTP in the EC. Our special interest in this cortex relates to its key position in the neocortex–hippocampus–neocortex circuit.

The occurrence of LTP in the EC after tetanic stimulation of afferent pathways as well as after direct rhythmic subthreshold membrane potential manipulation was investigated. Two types of *in vitro* preparations were used: (*i*) the adult guinea pig brain, isolated and maintained by arterial perfusion, and (*ii*) adult rat brain slices of the EC.

MATERIALS AND METHODS

Isolated Whole Brain *in Vitro* Preparation. Adult guinea pigs (150–200 g) were anesthetized with sodium pentobarbitol (60 mg/kg) and the brain temperature was reduced by intracardiac perfusion with ice-cold oxygenated modified Ringer's solution (see below). A craniotomy was performed and the brain was gently lifted from the skull. The brain, from the olfactory bulbs to the end of the medulla, was transferred to the perfusion chamber where one vertebral artery was cannulated (5, 6). Arterial perfusion (3–4 ml/min, determining a perfusion pressure of 70–100 mm Hg; 1 mm Hg = 133 Pa) was delivered by a peristaltic pump. The other vertebral artery, the carotids, and other leaking vessels were then ligated.

During this procedure the brain was superfused with ice-cold oxygenated Ringer's solution. The ionic composition of the superfusate and the perfusate was 126 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 15 mM dextrose, and 5 mM Hepes. A plasma expander [Dextran 70, 3% (vol/vol)] brought the perfusate to the proper osmotic pressure. After 1 hr at 15°C the preparation was slowly warmed to 32°C.

The piriform cortex (PC), which projects monosynaptically upon EC layers II and III (7, 8), was stimulated with bipolar twisted silver wires (insulated except for the tip). Field potentials were recorded with low-resistance (5–8 MΩ) 2 M NaCl-filled glass micropipettes. LTP was evoked by a set of 100-Hz pulse bursts (four shocks per burst) repeated five times per sec for 1–3 sec. This pattern, which mimics the θ-rhythm present in limbic structures, has been shown to be highly effective in inducing LTP (9). In some experiments the brain was perfused with a selective antagonist of *N*-methyl-D-aspartate (NMDA) receptors, DL-2-amino-5-phosphonovalerate (APV) (10) at 100 μM. APV reached the brain in 2–3 min. Experiments in the isolated brain were performed in the absence of picrotoxin.

Brain Slice Preparation. Horizontal brain slices (400 μm) were made from the retrohippocampal region of the adult rat by standard procedures (11). Slices were superfused with a 35°C solution containing 124 mM NaCl, 5 mM KCl, 1.2 mM $KHPO_4$, 2.6 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose (equilibrated with 95% O_2/5% CO_2). Intracellular recordings were obtained using 3 M potassium acetate-filled micropipettes. EC layer II neurons with a resting membrane potential of at least −60 mV and an input resistance >20 MΩ were analyzed. All experiments were carried out in the presence of the type A γ-aminobutyric acid ($GABA_A$)-receptor-blocker picrotoxin (100 μM).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EPSP, excitatory postsynaptic potential; EFP, extracellular field potential; LTP, long-term potentiation; EC, entorhinal cortex; NMDA, *N*-methyl-D-aspartate; APV, DL-2-amino-5-phosphonovalerate; PC, piriform cortex.

*Present address: Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, PQ, H35 2B4 Canada.

†To whom reprint requests should be addressed.

Bipolar stainless steel stimulating electrodes were placed on the molecular layer of the EC (layer I), 0.5 mm lateral to the recording electrode. Stimuli applied at 0.1 Hz activated associational fibers originating in the olfactory cortices (mainly the PC) (7) as well as intrinsic local projections. LTP was induced by a θ-pattern stimulation, similar to that used in the whole-brain preparation or by 10–20 sec of 5-Hz stimulation. In some experiments the superfusate contained APV and the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (12). The bath solution exchanged completely within 3 min.

RESULTS

LTP in the Isolated Brain. In all brains tested (n = 6), θ-patterned stimulation of the PC generated a significant increase in the amplitude of the monosynaptic extracellular field potentials (EFPs) in the EC. This potentiation could be followed for up to 4 hr. Laminar field-potential analysis demonstrated that PC stimulation elicits a monosynaptic surface negativity in the EC that is associated with a current sink at the level of the apical dendrites of the layer II and III cells and a corresponding current source in the depth of the cortex (8, 13). Recordings for the LTP experiments were made at 100–150 μm from the surface of PC, at the depth of the peak of the monosynaptic current sink.

An example of a long-lasting enhancement of the EFP in the PC–EC pathway is shown in Fig. 1*A* (top row and open squares in *B*). Control shocks delivered at 0.1 Hz to the PC elicited field responses with an average amplitude of 250 μV (trace a). After tetanic stimulation as described above, an early post-tetanic potentiation of the EFP was seen (trace b); the EFP amplitude decreased slightly within 3 min (trace c), to reach a stable potentiated level (trace d) where the EFP remained for up to 2 hr. Subtraction of the control and the enhanced EFPs (trace c-a) shows the potentiated response. In most of the experiments the LTP lasted as long as we kept the brain alive—in others it was transient, but lasted at least 2 hr.

As reported in other cortical structures, APV prevented the induction of LTP after tetanic stimulation of the PC in all isolated brain experiments (n = 4). As shown in Fig. 1*A* (lower row and solid squares in *B*), during APV perfusion (100 μM), PC tetanic stimulation induced only the post-tetanic potentiation (trace b'), which faded within 5 min (traces c' and d'). This result indicates that NMDA receptor activation is required for LTP induction in the PC–EC pathway. APV did not affect the nonpotentiated EFP.

APV also interfered with the expression of LTP. In Fig. 1*C*, after the initial post-tetanic potentiation, LTP amplitude continuously increased to reach a maximal amplitude within 10 min. A second tetanic stimulation (second arrow in *D*) generated further LTP (trace b). Perfusion with APV (bar in Fig. 1*D*) decreased the field potential to control value (trace c). This effect was reversible (trace d). The reduction in the potentiated EFPs in the presence of APV is shown in trace c-b. This result indicates that in the PC–EC pathway a NMDA-mediated mechanism is required for both the acquisition and expression of LTP.

LTP in EC Slices. LTP was elicited in EC layer II neurons by afferent stimulation, as shown in Fig. 2. This cell was identified as a layer II stellate cell by its subthreshold θ-like rhythmicity and the presence of strong delayed and anomalous rectification (15). A fast and a slow voltage-dependent component could be distinguished in the excitatory postsynaptic potentials (EPSPs) evoked by molecular-layer stimulation (Fig. 2*A*). All layer II cells investigated displayed a late voltage-dependent component.

The EPSPs in Fig. 2 *A* and *B* were elicited from three membrane-potential levels (resting potential, −62 mV). Note that the prominent late component in the EPSP elicited from a depolarized level (Fig. 2*A*, trace a) was reduced by membrane hyperpolarization (Fig. 2*A*, traces b and c). APV (50 μM) reduced the early peak of the EPSP and abolished the

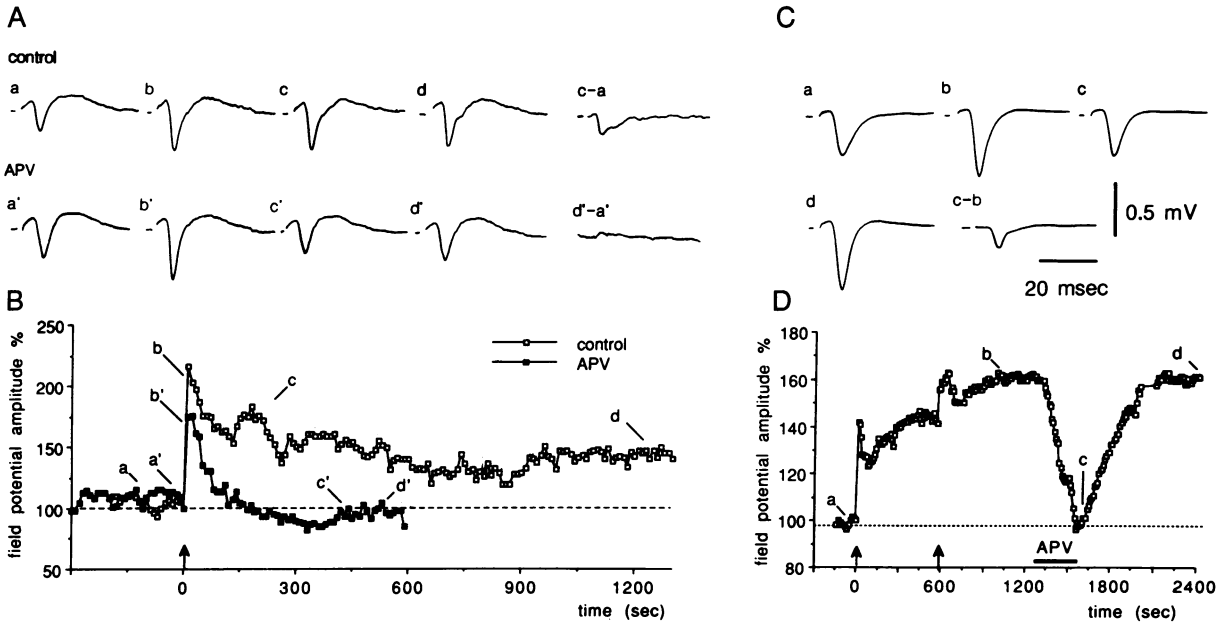

FIG. 1. EC LTP in the isolated whole-brain preparation at 32°C (*A*). Upper traces are averaged EC surface-field potentials (n = 5) before (trace a) and after (traces b–d) a "θ-like" tetanizing stimulus to the surface of the PC (arrow in the plot in *B*). Lower traces are the same as the upper traces but during APV perfusion. The traces on the far right of *A* isolate the field component induced by LTP in control conditions (trace c-a) and during 100 μm APV (trace d'-a'). (*B*) Changes with time in the normalized field-potential amplitude. Open squares, control; solid squares, during APV administration. (*C*) Effect of APV on the EFP after the induction of LTP. Control (trace a) and field potential (trace b) after two tetanic stimuli (arrowheads in *D*) that induced LTP. APV perfusion decreased the EFP to control values (compare traces a and c) after the LTP was induced and recuperated after APV washout (trace d). The reduction in the EFP amplitude during APV is shown in the subtraction trace c-b on the right. (*D*) Time course of LTP and its block by APV.

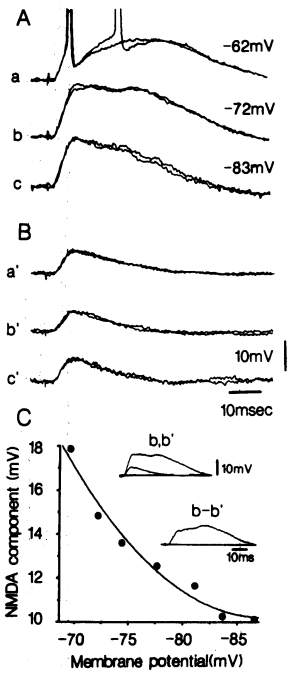

FIG. 2. NMDA-receptor-mediated component in EC layer II neurons. (*A*) In control Ringer's solution, EPSPs evoked by stimulating layer I (molecular layer) while recording at three membrane potential levels (traces a, b, and c; two traces shown at each level). The membrane potential was changed by injecting current pulses. Note the late component of the EPSP that develops upon depolarization. (*B*) EPSP obtained at the same three levels of membrane potential as in *A* but in the presence of 50 μm APV. The APV abolished the late component. (*C*) Amplitude of the APV-sensitive component (NMDA receptor mediated) of the EPSP as a function of membrane potential. (*Upper Inset*) Superposition of the averaged middle traces b (control) and b' (APV). The EPSP obtained by subtracting the APV-resistant (trace b') from the control EPSP (trace b) is shown (*Lower Inset*).

late component (Fig. 2*B*; see also Fig. 2*C Insets*). The APV-sensitive component of the EPSP as a function of membrane potential (Fig. 2*C*) clearly shows the voltage-dependent properties of the slow component of the EPSP and its enhancement with depolarization. The block of this "anomalous" voltage-dependent property by APV indicates that it is dependent on NMDA-receptor activation. Application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (20 μM) abolished the APV-insensitive component of the EPSP (n = 2, data not shown), indicating that this is mediated by quisqualate/kainate receptors (14).

θ-Pattern 10–20 sec 5-Hz single-shock afferent stimulation resulted in a long-lasting increase in EPSP amplitude (Fig. 3). Control shocks evoked constant amplitude EPSPs (Fig. 3*A*, traces a). After test stimulation (5 Hz for 10 sec), the EPSP amplitude increased (Fig. 3*A*, trace b) but decreased in <2 min (Fig. 3*A*, trace c). Thereafter, the amplitude increased again and a prominent late component developed (Fig. 3*A*, trace d). Six minutes after the test stimulation the EPSP was maximal and a spike was elicited (Fig. 3*A*, trace e). The EPSP remained at this potentiated value for the length of the recording period (35 min). In Fig. 3*A* (traces a and e), control (trace a) and potentiated EPSPs (trace e) have been superimposed to illustrate the changes in the EPSP after potentiation. Note that the augmentation of the late component of the EPSP was not accompanied by an input resistance change as illustrated by the voltage responses to the hyperpolarizing current pulses in traces a to e. In all the cells tested (n = 5) tetanic-induced changes persisted for the duration of the observation period (from 15 min to 2 hr).

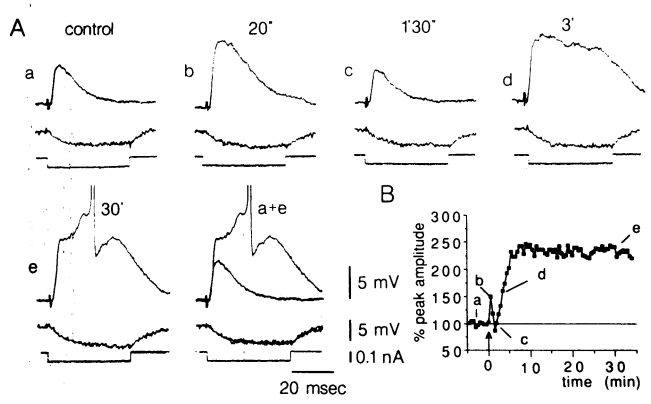

FIG. 3. Time course of LTP of EPSPs induced by tetanic afferent fiber stimulation. (*A*) Records a through e show potentials recorded intracellularly at the times indicated. Upper records show the intracellularly recorded EPSP; lower records show the membrane response to a hyperpolarizing current pulse used to monitor the input resistance. At time 0 repetitive (arrow in *B*) 5-Hz single-shock stimulation was applied for 10 sec to the EC molecular layer at the same intensity as during the control period. The superposition of traces a and e shows the increase in EPSP amplitude induced by the tetanic stimulation. (*B*) Normalized peak EPSP amplitude with respect to time. Letters correspond to EPSPs. In this plot and in those in Figs. 4 and 5 when the cell fired, the peak EPSP amplitude was measured at the firing level. Each point is the mean of three consecutive measurements.

Non-Hebbian LTP. EC layer II neurons have been shown to have intrinsic oscillation properties that may play an important role in the genesis of the θ rhythm. These cells display a Na-dependent 2- to 7-mV subthreshold membrane potential oscillation in the θ frequency range (15). Because it has been suggested that θ rhythmicity may underlie synaptic plasticity (9), we designed a series of experiments to determine whether postsynaptic manipulations could potentiate synaptic transmission in the absence of evoked synaptic input. In these experiments control EPSPs were evoked by white matter stimulation. The intracellular conditioning stimulus comprised of 20-sec bursts of 100-msec subthreshold (10–20 mV), depolarizing current pulses delivered at 5 Hz. This conditioning stimulus was not paired with white matter stimulation and was usually applied from 5- to 10-mV hyperpolarization from rest to prevent spike activation. Intracellularly induced increases in synaptic strength, developing within 30 sec from the intracellular stimulation, were observed in 16 of the 24 cells tested. In half of these cells the increased synaptic strength persisted for the duration of the observation periods (30 min to 3 hr). A shorter potentiation of at least 10 min was seen in the other 8 neurons.

Fig. 4 illustrates postsynaptically induced LTP. Control EPSPs were evoked by white matter stimulation (Fig. 4*A*). The intracellular conditioning stimulus was then applied from a −70-mV membrane potential from a resting potential of −60 mV (Fig. 4*B*). Twenty seconds after the intracellular train, the first test white matter stimulus evoked an enhanced EPSP (Fig. 4 *C* and *G*), which soon reached firing threshold (Fig. 4*D*). In this cell the synaptic strength remained potentiated for the recording period of almost 3 hr (Fig. 4 *F* and *G*). The input resistance of these cells during this paradigm remained constant throughout the experiments.

In agreement with the results obtained in the isolated-brain preparation, afferent fiber tetanic stimulation in the presence of APV (50 μM) did not induce any significant LTP (n = 3). In addition administration of APV prior to intracellular direct stimulation prevented the increase in EPSP amplitude (n = 4). These results clearly indicate that the activation of NMDA receptors is necessary for the induction of both Hebbian and non-Hebbian LTP in the EC.

In contrast to the hippocampus, where a selective increase in non-NMDA component of the EPSP has been observed with NMDA-dependent LTP (16, 17, 18), our results indicated that in the EC the non-NMDA-mediated component of the EPSP remained unchanged after intracellular induced LTP. The NMDA component appears, in consequence, to be responsible for the observed long-lasting increase in synaptic strength in the EC. This is shown in Fig. 5 in which the non-NMDA component of the EPSP was examined before and after intracellularly induced LTP. Two control EPSPs, in which the late component was very apparent, are superimposed in Fig. 5*A*. This component was blocked by APV (50 μM) revealing the non-NMDA component (Fig. 5*A*, trace b, and *B*). The NMDA component returned 6 min after superfusion with the control solution (Fig. 5 *A*, trace c, and *B*). Five

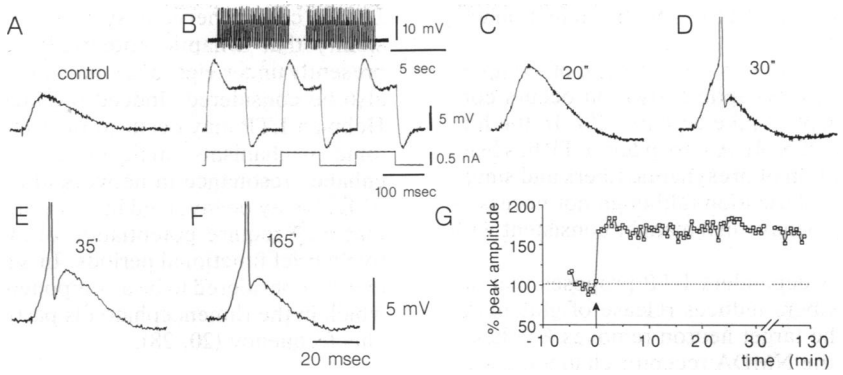

FIG. 4. Time course of LTP of EPSPs induced by postsynaptic subthreshold rhythmic membrane depolarizations. (*A*) Control EPSP. The traces in *A* and *C–F* were recorded at the times indicated after rhythmic depolarization of the cell. *B* illustrates the characteristics of the postsynaptic stimulation. (*D*) Note the powerful delayed rectification in the upper trace was broken to illustrate the beginning and end of the 30-sec stimulus train. (*G*) Normalized peak EPSP amplitudes during the potentiation as a function of time. Arrow indicates time of postsynaptic stimulation.

minutes after the recording in Fig. 5*A*, trace c, the intracellular conditioning burst of stimuli was applied. The first test EPSP, evoked 20 sec after the intracellular stimulation, was clearly potentiated for the next 15 min (Fig. 5 *A*, trace d, and *B*). At that time the slice was again superfused with APV (50 μM) to isolate the non-NMDA component of the EPSP (Fig. 5*A*, trace e) for comparison with the same component before potentiation (trace b). Trace f shows the recovery of the NMDA component of the EPSP. The non-NMDA components of the EPSP before and after potentiation have been superimposed in trace b + e. It is clear that the non-NMDA component did not change during LTP, indicating that the NMDA component is indeed responsible for the potentiation induced by intracellular stimulation. Similar results were found in three other experiments.

DISCUSSION

We have demonstrated the presence of LTP in the EC after tetanic stimulation of the PC using an isolated-brain preparation. Intracellular recordings from brain slices demonstrated that this LTP is induced in EC layer II neurons by afferent fiber tetanic stimulation and by subthreshold membrane potential stimulation of the postsynaptic cell. These results indicate that important changes in synaptic efficacy related to the functions of the hippocampal circuits may be taking place in neurons in EC layer II. These changes will deeply affect the activity in the hippocampal "trisynaptic circuit" for which stellate cells provide the afferent drive by the "perforant path" (19). LTP in the EC after stimulation of afferent pathways (including the PC–EC pathway) has been reported (4, 20) but the underlying cellular mechanism was not investigated.

Our pharmacological studies in the isolated-brain preparation associated NMDA receptors with the induction as well as the expression of LTP in the PC–EC pathway. APV always prevented LTP induction by PC stimulation, indicating that a NMDA-mediated mechanism is required in the EC, as has been shown in the hippocampus (for review, see ref. 3) and other cortical structures (21, 22).

In the hippocampus, blocking NMDA receptors with APV did not abolish the expression of LTP once it was established (17, 18). In the PC–EC pathway, we found, however, that the amplitude of the potentiated monosynaptic evoked potential was reduced to control levels after perfusion with APV, suggesting that NMDA receptor activation is not only involved in the induction of LTP but also in its expression.

Our *in vitro* intracellular recordings from EC layer II neurons revealed a fast and a slow component in the EPSP evoked by electrical stimulation of the EC molecular layer that were abolished by APV, indicating its mediation through NMDA receptor (10). This finding was further supported by the fact that the APV-sensitive components displayed voltage-dependent properties characteristic of NMDA-receptor-mediated events (23, 24). It seems reasonable to assume, therefore, that the NMDA-mediated components of the EPSP observed in the slice preparation provide the basis for the LTP observed in the isolated-brain preparation. In fact, and in full agreement with this interpretation, tetanic stimulation of the EC molecular layer [where most afferent PC originating fibers terminate (7)] also resulted in a long-lasting

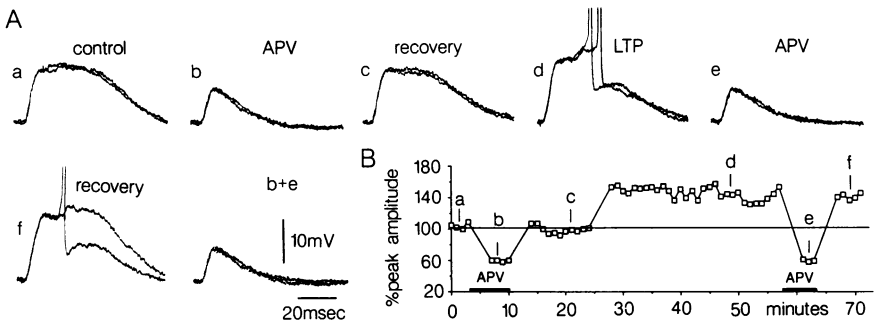

FIG. 5. NMDA-receptor-mediated component of the EPSP selectively increased after intracellularly induced LTP. (*A*) Records show potentials (two superimposed traces) recorded intracellularly at various times through the experiment as indicated in *B*. Traces a through c show the effect of APV on the control EPSP. In trace d the EPSP is potentiated by an intracellular train of subthreshold 100-msec depolarizing current pulses delivered at 5 Hz. Traces e and f show the effect of APV on the potentiated EPSP and the recovery after washout of APV, respectively. The superposition of the APV-resistant component in control and potentiated is illustrated in trace b + e. (*B*) Normalized amplitudes of the EPSPs plotted against the time through the recording shown in *A*.

increase in the amplitude of the EPSP, and this induction was found to be APV sensitive.

According to Hebb's hypothesis, the strength of synapses may be enhanced when presynaptic activation occurs concomitantly with postsynaptic spike activity (25). In the hippocampus, the induction of NMDA-dependent LTP has been shown to require stimulation of presynaptic fibers and simultaneous postsynaptic depolarization (although not necessarily spiking) (3); i.e., they behave in a manner consistent with Hebb's postulate.

One model for NMDA-dependent LTP proposes that activation of presynaptic fibers induces release of glutamate, which by depolarizing the target neuron removes a voltage-dependent Mg block of the NMDA receptor channel, allowing Ca to enter through this channel, which serves as a trigger for LTP (3, 26).

The above pseudo-Hebbian model for LTP may also apply to the induction of NMDA-dependent LTP in the EC after afferent fiber stimulation reported here. However, since our results show that NMDA-dependent LTP can also be obtained after postsynaptic rhythmic subthreshold membrane potential manipulation, other possibilities must be considered. The latter finding invokes an entirely non-Hebbian mechanism, since in this paradigm the presynaptic and postsynaptic elements were never stimulated simultaneously.

The finding that intracellularly induced LTP was never obtained in the presence of APV indicates that NMDA receptor activation is essential in this induction. Further, it implies the presence of sufficient extracellular glutamate to activate NMDA receptors in the absence of afferent stimulation, as reported for pyramidal cells in hippocampal slices (27). In agreement with that report, we also observed a decrease in the excitability in EC layer II neurons to direct stimulation during bath application of APV.

Because in EC stellate cells neither hyperpolarizing current pulses nor maintained subthreshold depolarizations potentiated synaptic efficacy, a special mechanism must be at work in the non-Hebbian LTP. We propose that in addition to relieving the voltage-dependent block of the NMDA channel, rhythmic depolarizations can lock into a constructive resonance with the intrinsic oscillations present in these neurons (14). This mechanism would allow peak depolarizations to reach remote dendritic postsynaptic sites, very effectively generating a pulsatile entry of Ca through NMDA channels opened by background glutamate present in the extracellular medium. By contrast steady depolarization would have a less prominent remote depolarizing effect due to the powerful delayed rectification present in these cells (Fig. 4*B*). We must also consider the possible existence of an intermediate step in the potentiation of the NMDA channel, which would display time- or calcium-dependent inactivation, that is, an underlying chemical oscillator that resonates with the rhythmic entry of Ca into the cell.

From a functional point of view intracellularly induced LTP in EC layer II neurons leads to a selective increase in the NMDA-mediated component of the EPSP (Fig. 5). This suggests that the enhancement of synaptic transmission during intracellular-induced LTP is exclusively due to a postsynaptic modification.

The finding that LTP in the EC occurs in Hebbian as well as non-Hebbian paradigms raises the question of the role of LTP in central nervous system function. Although the possibility that synaptic potentiation is related to memory is presently under vigorous study, other functions for LTP must also be considered. Indeed the fact that Hebbian and non-Hebbian LTP may occur in the same cell, utilizing the same ionic mechanisms, indicates that LTP may also serve to enhance resonance in networks that fire rhythmically. That is, LTP may be involved in many states—such as attention—that may require potentiation of synaptic efficacy for relatively brief functional periods. In addition non-Hebbian LTP may be considered to be a component in "epileptic kindling," which in the rhinencephalon is particularly sensitive to stimulus frequency (20, 28).

This research was supported by Grant NS13742 from the NINCDS and Italian CNR Grant 2042856 to M. de C.

1. Bliss, T. V. & Lomo, T. (1973) *J. Physiol. (London)* **232**, 331–356.
2. Ramon y Cajal, S. (1904) *Histologie du Systeme Nerveux de L'Homme et des Vertebrates* (Instituto Ramon y Cajal, Madrid).
3. Gustafson, B. & Wigstrom, H. (1988) *Trends NeuroSci.* **11**, 156–162.
4. Tyler, T. Y. & Discenna, P. (1987) *Annu. Rev. Neurosci.* **10**, 131–161.
5. Llinás, R. & Mühlethaler, M. (1988) *J. Physiol. (London)* **404**, 215–240.
6. Llinás, R., Mühlethaler, M. & Walton, K. (1989) *J. Physiol. (London)* **414**, 16.
7. Krettek, J. E. & Price, J. L. (1977) *J. Comp. Neurol.* **172**, 723–752.
8. Van Groen, T., Lopes de Silva, F. H. & Wadman, W. J. (1987) *Exp. Brain Res.* **67**, 615–622.
9. Larson, J., Wong, D. & Lynch, G. (1986) *Science* **232**, 985–988.
10. Davies, J. P., Francis, A. A., Jones, A. W. & Watkins, J. C. (1981) *Neurosci. Lett.* **21**, 77–81.
11. Llinás, R. & Sugimori, M. (1980) *J. Physiol. (London)* **305**, 197–213.
12. Honoré, T. (1988) *Eur. J. Pharmacol.* **151**, 161–162.
13. Boeijinga, P. H. & Van Groen, T. (1984) *Exp. Brain Res.* **57**, 40–48.
14. Blake, J. F., Brown, M. W. & Collingridge, G. L. (1988) *Neurosci. Lett.* **89**, 186–192.
15. Alonso, A. & Llinás, R. (1989) *Nature (London)* **342**, 175–177.
16. Zalutsky, R. A. & Nicoll, R. A. (1990) *Science* **248**, 1619–1624.
17. Muller, D. & Lynch, G. (1988) *Science* **242**, 1694–1697.
18. Kauer, J. A., Malenka, R. C. & Nicoll, R. A. (1988) *Neuron* **1**, 911–917.
19. Andersen, P., Holmquist, B. & Voorhoeve, P. E. (1966) *Acta Physiol. Scand.* **66**, 448–460.
20. Racine, R. J., Milgram, N. W. & Hafner, S. (1983) *Brain Res.* **260**, 217–231.
21. Artola, A. & Singer, W. (1987) *Nature (London)* **330**, 649–652.
22. Kimura, F., Nishigori, A., Shirokawa, T. & Tsumoto, T. (1989) *J. Physiol. (London)* **414**, 125–144.
23. Nowak, L., Bregestowski, P., Asher, P., Herbet, A. & Prochiantz, A. (1984) *Nature (London)* **307**, 462–464.
24. MacDonald, J. F., Porietis, A. V. & Wojtowicz, J. M. (1982) *Brain Res.* **237**, 248–253.
25. Hebb, D. O. (1949) *The Organization of Behaviour* (Wiley, New York).
26. Malenka, R. C., Kauer, J. A., Zucker, R. S. & Nicoll, R. A. (1988) *Science* **242**, 81–84.
27. Sah, P., Hestrin, S. & Nicoll, R. A. (1989) *Science* **246**, 815–818.
28. Cain, D. P. (1989) *Trends NeuroSci.* **12**, 6–10.