Evidence that associative interactions between synapses during the induction of long-term potentiation occur within local dendritic domains

(long-term depression/neuronal plasticity/dentate gyrus)

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ABSTRACT   The present study evaluates whether the associative interactions between synapses that lead to long-term potentiation and depression (LTP and LTD) can occur between spatially segregated synapses of the medial and lateral temporodentate pathway of the rat. Coconditioning of crossed and ipsilateral pathways resulted in LTP of the crossed system only when the current sinks of the two conditioned pathways overlapped sufficiently. Likewise, conditioning of an ipsilateral pathway alone resulted in LTD of the crossed pathway only when those current sinks overlapped sufficiently. These observations support the idea that associative events that lead to LTP or LTD can be restricted to a local dendritic domain. The postsynaptic cell can therefore serve as more than one unit of integration for synaptic modification.

Long-term potentiation (LTP) is characterized by a persistent increase in synaptic efficacy following brief high-frequency stimulation of certain afferent pathways within the central nervous system (1–3). There is considerable evidence that LTP results from associative interactions derived from concurrent activity in a sufficient number of excitatory afferents that converge upon a given postsynaptic cell. For example, McNaughton *et al.* (4) demonstrated that high-frequency activation of a pathway leads to LTP only if a large number of the axons in that pathway are coactivated. Furthermore, weak excitatory inputs that do not exhibit LTP when stimulated alone do exhibit LTP when coactivated with a somewhat stronger input (5, 6). The associative property of LTP, and the fact that it can last for weeks, makes it an attractive candidate mechanism for associative information storage in the central nervous system.

Early studies suggested that the associative nature of LTP depended upon the discharge of the postsynaptic cell (4). This idea stemmed primarily from a literal interpretation of Hebb (7), who hypothesized that the strength of synapses would increase when their activity was repeatedly associated with postsynaptic cell firing. Hebb's hypothesis seemed to account for the associative nature of LTP because the minimum intensity of stimulation that was required to induce LTP was near the threshold for postsynaptic cell firing (4).

The proposed association between the induction of LTP and cell firing was brought into question by several subsequent studies. For example, LTP can be induced when cell firing has been blocked by activation of inhibitory afferents (8) or by injection of hyperpolarizing current into the postsynaptic cell (9). However, more recent studies have revealed that postsynaptic injection of a hyperpolarizing current can interfere with the induction of LTP in afferents that terminate close to the site of the current injection (10). One interpretation of these findings is that the induction of LTP depends on sufficient dendritic depolarization near the site of the active synapses rather than on depolarization due to cell firing.

The present study evaluates the hypothesis that associative events that lead to LTP depend upon the level of dendritic depolarization at the site of the active synapses. The study takes advantage of the topographic organization of pathways from the entorhinal cortex to the dentate gyrus (the temporodentate pathways). Projections from the lateral portions of the entorhinal cortex (LEC) terminate on distal dendrites, whereas projections from the medial entorhinal cortex (MEC) terminate more proximally (11, 12). In addition, the temporodentate pathways have ipsilateral and crossed components (12). The ipsilateral pathways comprise at least 90% of the synapses in the entorhinal terminal zone, while the crossed pathways comprise only 5% of the synapses (13). Whereas high-frequency conditioning of an ipsilateral pathway results in LTP (4), high-frequency conditioning of the crossed pathway does not (5). LTP can, however, be induced in the crossed pathway if it is coconditioned with a converging ipsilateral pathway (5). In this respect, LTP of the crossed pathway depends upon associative interactions with the ipsilateral pathway. Further, when an ipsilateral pathway is conditioned alone, a long-term depression (LTD) is observed in the converging crossed pathway (5, 14, 15). In this sense, LTD is also an associative process.

If the associative interactions responsible for LTP and LTD can be restricted to local dendritic domains, then such interactions should be maximal when coconditioned afferents converge upon the same proximodistal segment of a given dendrite. Alternatively, if spatial constraints do not exist, then spatially segregated afferents should interact equally well, irrespective of where along the dendrite they terminate.

MATERIALS AND METHODS

Acute neurophysiological methods were similar to those of previous studies (5, 14). Long Evans hooded rats (200–350 g) were anesthetized with chloralose/urethane (55 and 400 mg/kg, respectively). Glass micropipette recording electrodes were positioned stereotaxically in the hilus of the dentate gyrus in order to record evoked potentials resulting from activation of the temporodentate pathways (Fig. 1). Stimulating electrodes were positioned bilaterally in the MEC and LEC. These electrodes were used to stimulate the medial and lateral pathways, which project, respectively, to

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Abbreviations: LTP, long-term potentiation; LTD, long-term depression; MEC, medial entorhinal cortex; LEC, lateral entorhinal cortex; pEPSP, population excitatory postsynaptic potential; ANOVA, analysis of variance; CSD, current source density.
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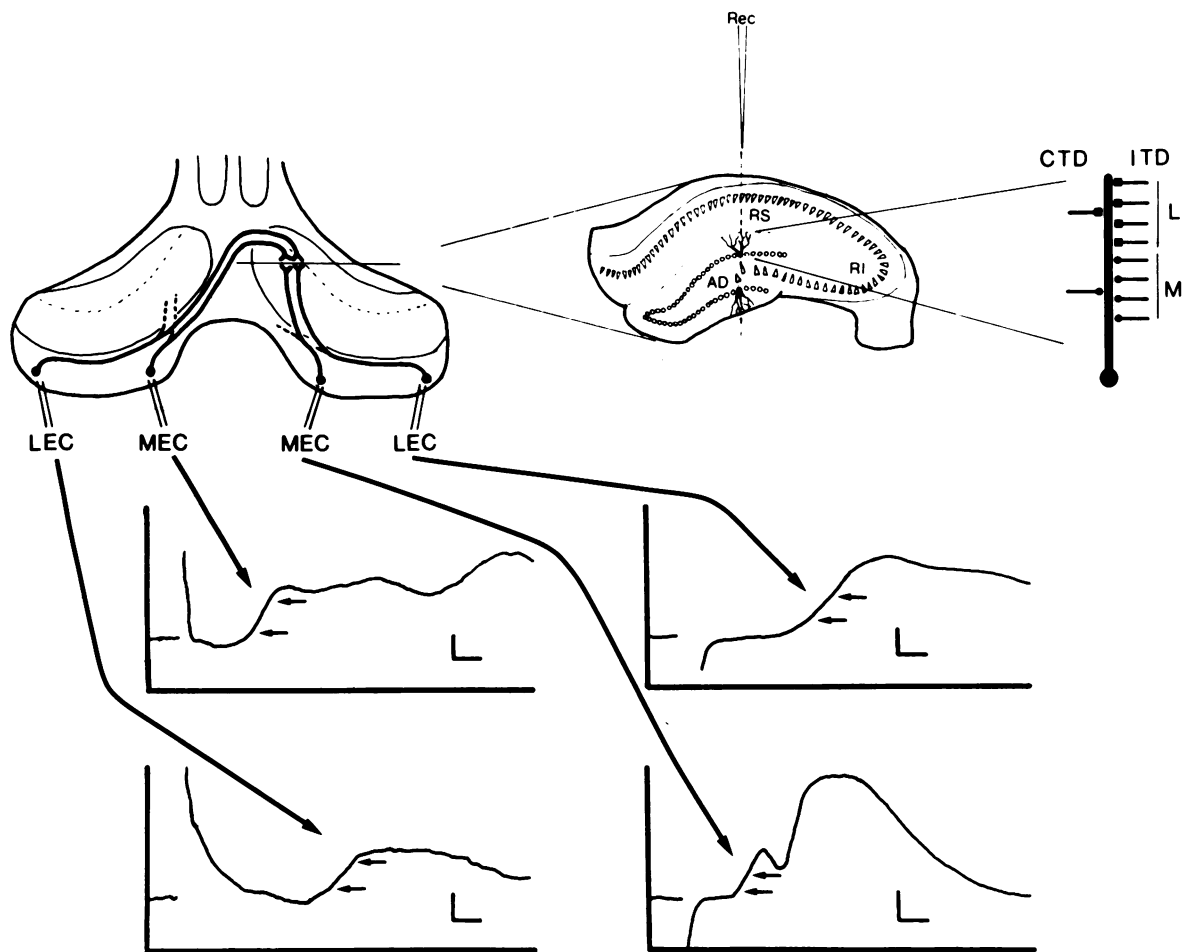*Proc. Natl. Acad. Sci. USA 85 (1988)* 2369

FIG. 1. Schematic representation of the rat hippocampal formation and electrode placements. Also schematically illustrated is the relationship along the dendritic tree of the synapses activated by the medial and lateral stimulating electrodes. The vertical scale is 0.2 mV for contralateral responses and 2.0 mV for ipsilateral responses while the horizontal scale is 1 msec (scale bars are insets in evoked-responses drawings). ITD, ipsilateral temporodentate pathway; CTD, contralateral temporodentate pathway; L, lateral; M, medial; RS, regio superior; RI, regio inferior; AD, area dentata; Rec, recording electrode.

middle and distal dendrites of dentate granule cells (Fig. 1). Stereotaxic coordinates for the stimulating electrodes were 3.0 mm lateral to the midline and 1.2 mm anterior to the transverse sinus for the MEC electrode and 5.5–6.5 mm lateral to the midline and 1.4–1.5 mm anterior to the sinus for the LEC electrode. Stimulating electrode depth was adjusted to achieve the maximal evoked potential in the dentate gyrus during low-frequency test stimulation. The stimulating electrodes were affixed to the skull with dental cement before data collection began.

For testing, stimulation intensity was adjusted for each pathway so that response amplitudes in the ipsilateral pathways were about 70% of maximum amplitude, which in our experience is very close to threshold for LTP. Response amplitudes in the crossed pathways were <1 mV. Baseline response amplitude was determined over a 15-min period, after which conditioning stimulation was delivered to the pathways in various combinations (see legend of Fig. 3). Each set of conditioning trains (8 pulses at 400 Hz, delivered 8 times at 10-sec intervals) was followed by a 15-min data-collection period. During each data-collection period, stimulation was delivered to each pathway once every 30 sec in an alternating fashion. The initial slope (mV/msec) of the population excitatory postsynaptic potentials (pEPSPs) evoked by each pathway was determined (see Fig. 1). The change in response amplitude following each manipulation was calculated by dividing the average of the 20 responses of the final 10 min of the collection period following the manipulation by

the average of the 20 responses preceding the manipulation. The data were then expressed as percent change from preceding baseline.

Fifteen minutes after delivery of the final set of conditioning trains, the recording electrode was advanced through dorsal and ventral blades of the dentate gyrus in 20-μm steps, collecting four evoked responses from each pathway at each site. The four responses were averaged and the initial slope of the pEPSP was determined at a constant latency from the stimulation pulse at a time prior to cell firing. A current source density (CSD) was constructed by taking the second derivation of the field potential profile, using the three-point formula of Freeman and Nicholson (16). Within a defined part of the brain, a CSD analysis of evoked field potentials can be used to indicate the locations in which a net current flows into or out of neuronal elements (16). A current sink is said to exist in areas where a net current flow is into neuronal elements (areas of cell depolarization). These areas can be said to represent synaptic activation under the circumstances of this study (17).

The percent overlap between current sinks of different pathways was determined by (*i*) calculating the percent of the area under the curve corresponding to the current sink generated by the ipsilateral pathway that overlapped with the current sink generated by the crossed pathway and then (*ii*) determining the percent of the area under the curve corresponding to the current sink generated by the crossed pathway that overlapped with the current sink generated by

the ipsilateral pathway. The final overlap value was the average of the two above overlap values.

The paired *t* tests and two-way general linear model analyses of variance (ANOVAs) were executed with an Apple IIe computer using the program APPSTAT (Statsoft, Tulsa, OK).

RESULTS

The CSD analysis was used to determine the apparent overlap between medial and lateral pathways for each experiment. Fig. 2 shows examples of CSD profiles of pEPSPs generated by stimulation of the different subdivisions of the temporodentate system. Such plots allow an examination of the interactions between afferents that converge upon the same proximodistal segment of the dendritic tree (Fig. 2 *Upper*) or afferents that terminate at different segments (Fig. 2 *Lower*). As expected, the pathways from similar mediolateral areas of the two cortices showed considerable overlap (Table 1).

The CSD analysis was important, since the extent of overlap depends critically upon stimulating-electrode location and stimulus intensity. For this reason, there was some variability in the extent of CSD overlap from experiment to experiment. Thus, for the present report, we limited our evaluation to preparations where medial and lateral pathways exhibited no more than 50% overlap of current sinks (Table 1). Preparations where pathways exhibited a greater degree of overlap will be considered in a separate publication.

The principal results of this study are illustrated in Fig. 3. The upper bar graphs illustrate the average changes in the amplitude of pEPSPs evoked by the crossed pathway from the LEC. The lower bar graphs illustrate the average changes in the amplitude of the pEPSPs evoked by the crossed pathway from the MEC. The illustrations at the

Table 1. Percent overlap between current sinks of interacting pathways

Contralateral	Ipsilateral	
	Lateral	Medial
Lateral ($n = 9$)	94 ± 12	11 ± 12
Medial ($n = 5$)	18 ± 20	90 ± 7

Percent overlap of current sinks from medial and lateral temporodentate pathways. Overlap is expressed as a percent $\pm$ 1 SD. Percent overlap was calculated as described in *Materials and Methods*.

bottom of the figure indicate which pathways were conditioned prior to each data-collection period.

As illustrated in the leftmost set of bars (bars 1), coconditioning of the lateral pathway resulted in LTP of the lateral crossed pathway and no change in the response of the medial crossed pathway. Similarly, as illustrated by bars 2, coconditioning of both medial pathways led to LTP of the medial crossed pathway, while the response amplitude of the nonconverging lateral crossed pathway was unaffected.

As illustrated by bars 3, conditioning of the lateral pathway from one hemisphere induced LTD in the converging lateral crossed pathway but not in the medial crossed pathway, which terminated at a different level of the dendrite. Similarly, conditioning of the medial pathway resulted in LTD of the converging medial crossed pathway (bars 4). Because the third and fourth manipulations leave each crossed pathway in a depressed state, LTP can again be evaluated (5, 14).

Coconditioning of ipsilateral and crossed pathways terminating at different levels of the dendrite did not induce significant LTP in either crossed pathway (bars 5 and 6). The absence of LTP in response to this manipulation is not likely to be due to prior induction of LTD (by manipulations 3 and
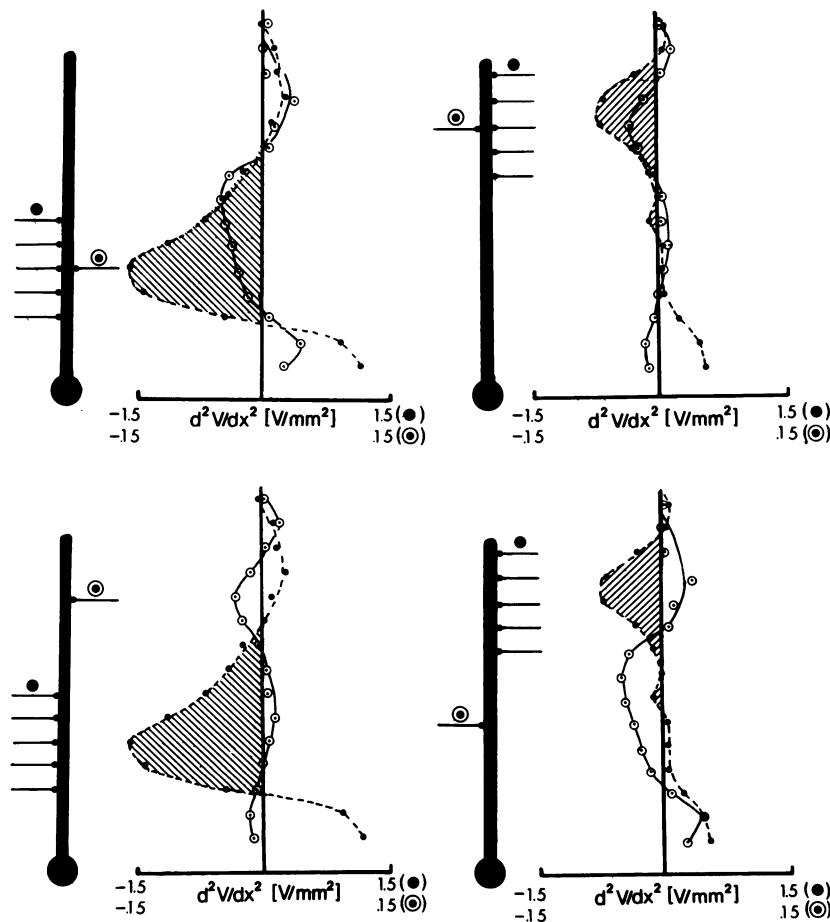

FIG. 2. Current source density analysis of the pEPSP evoked from the medial and lateral pathways. (*Upper*) The overlap in current sinks between both medial or both lateral pathways is indicated. (*Lower*) Segregation between medial and lateral pathways is indicated. In each graph, current sinks of ipsilateral inputs are shaded (hatching). The filled figure to the left of each graph represents the approximate location of the granule-cell soma and dendrite. The thin lines perpendicular to the schematic dendrites represent dense ipsilateral pathways (filled circles) and sparse contralateral pathways (circles with a dot).
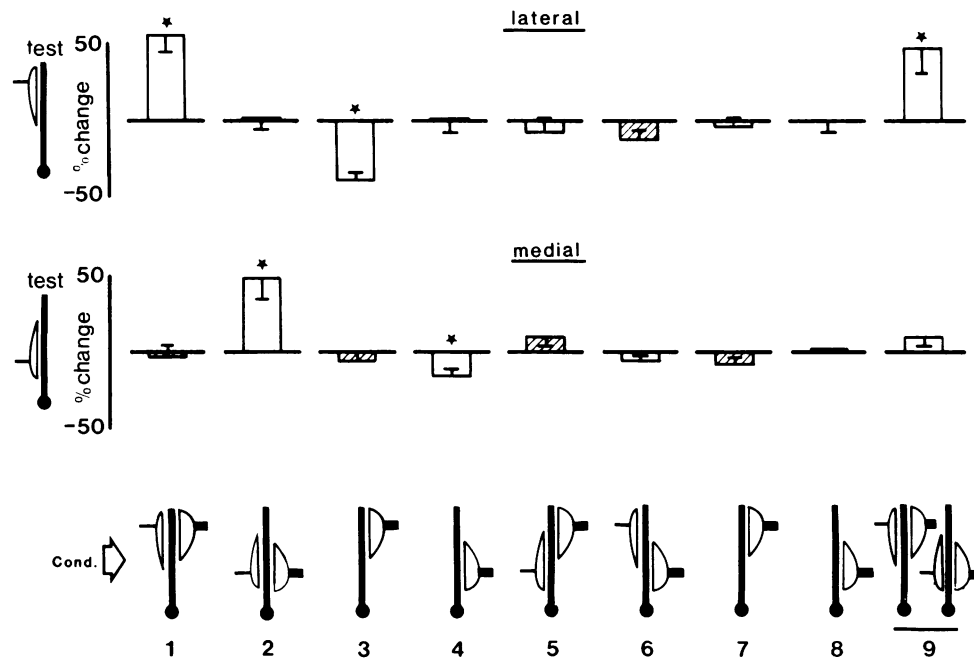
FIG. 3.   Changes in the amplitude of responses of lateral and medial crossed pathways after various conditioning manipulations. The upper set of bar graphs illustrates the changes in response amplitude that were observed in the crossed pathways from the LEC ($n = 9$). The lower set of bar graphs illustrates the changes in response amplitude that were observed in the crossed pathways from the MEC ($n = 5$). The conditioning manipulations are schematically illustrated by the numbered stick figures below the bar graphs to which each manipulation corresponds. The circle of each stick figure represents the cell soma. The shaft of the figure represents the dendritic tree. The off-center semicircles drawn next to the schematic dendritic tree represent the inputs that were conditioned. The sparse contralateral afferents are represented by the thin line and small semicircle, whereas the dense ipsilateral set of afferents is represented by the thick line and large semicircle. Fifteen minutes elapsed between each manipulation except in manipulation 9, when the two sets of conditioning trains were separated by only 1 min. The units of the y axis are percent of previous baseline. The error bars are standard errors of the mean. A star indicates that the group was different from the immediately preceding baseline ($P < 0.005$; paired t test). Actual P values can be found in the text.

4), since previous studies have shown that LTP and LTD can be elicited repetitively (5).

Bars 7 and 8 indicate that a repetition of the conditioning that led to LTD in each pathway (bars 3 and 4) resulted in no further depression, despite the fact that coconditioning of pathways that terminate on different segments of the dendrite had occurred in the interim. The ninth and final manipulation in Fig. 3 increased the amplitudes of the crossed responses so as to facilitate measurement of the CSD.

This study evaluated two treatment effects on synaptic modification: (*i*) the effect of current sink overlap and (*ii*) the effect of stimulating-electrode placement (medial versus lateral). A two-way ANOVA evaluated these two effects on LTP and LTD.

As illustrated in Fig. 4*A*, potentiation was greatest when contralateral and ipsilateral pathways terminated at the same dendritic level [$F = 24.6$; $P = 0.0005$; degrees of freedom (df) = 1,12; manipulations 1 and 2 vs. 5 and 6]. Since the extent of LTP was comparable in medial and lateral pathways ($F = 0.93$; $P = 0.3564$; $df = 1,12$; manipulations 2 and 5 vs. 1 and 6, respectively), the medial and lateral groups were pooled for comparison to baseline by a paired t test. When ipsilateral and contralateral pathways terminating at the same dendritic level were coactivated, the crossed pathway exhibited LTP ($P = 0.0006$, $n = 14$). LTP was not observed when pathways that terminated at different dendritic levels were coactivated ($P = 0.6128$, $n = 14$). LTP values from manipulation 9 were not included in the analysis because only 1 min separated the conditioning trains to the medial and lateral pathways.

As illustrated in Fig. 4*B*, depression was greatest when contralateral and ipsilateral pathways terminated at the same dendritic level ($F = 44.16$; $P = 0.0001$; $df = 1,12$; manipulations 3 and 4 vs. 2 and 3). The ANOVA also revealed a

significant interaction effect when evaluating LTD in the medial and lateral pathways ($F = 19.31$; $P = 0.0011$; $df = 1,12$). Analysis of simple main effects indicated a difference
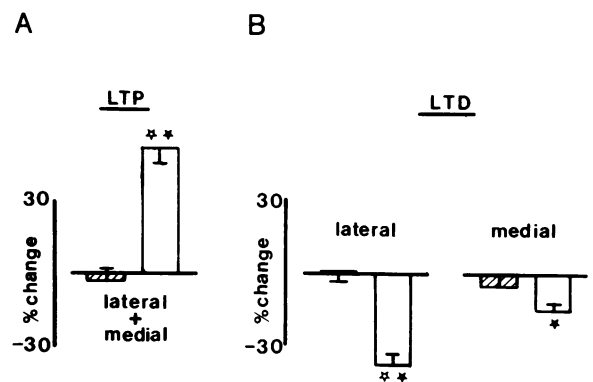

FIG. 4.   (*A*) Summary of LTP in contralateral pathways. Since no interaction effects were observed in the two-way ANOVA ($F = 2.06$; $P = 0.1740$; $df = 1,12$), medial and lateral pathways were pooled for all analyses. The hatched bar shows the average changes following manipulation 5 (medial pathway) and manipulation 6 (lateral pathway). The open bar shows the average changes following manipulation 2 (medial pathway) and manipulation 1 (lateral pathway). The y axis is percent change from previous baseline. Solid star indicates that a group was different from baseline ($P = 0.0006$). Open star indicates LTP in the open bar graph was greater than in the hatched bar graph of the 0–50% overlap group ($P = 0.0005$). (*B*) Average changes in response amplitude in lateral pathways following manipulations 2 and 3 (hatched and open bars, respectively) and in medial pathways in response to manipulations 3 and 4 (hatched and open bars, respectively). A solid star indicates that a group was different from baseline ($P < 0.05$). Open star indicates that LTD in the open bar was greater than in the neighboring hatched bar ($P < 0.05$). See text for exact P values.

between the medial and lateral pathways in manipulations expected to show LTD ($P = 0.0017$; $df = 1,24$), even though no difference was found between the medial and lateral groups in manipulations that did not lead to LTD ($P = 0.3370$; $df = 1,24$; see ref. 18).

Because of the significant interaction, medial and lateral pathways were evaluated separately for LTD (Fig. 4B). LTD was observed when the inactive crossed and conditioned ipsilateral pathways terminated at the same dendritic level (medial, $P = 0.0096$, $n = 5$, manipulation 4; lateral, $P = 0.0001$, $n = 9$, manipulation 3). LTD was not observed when ipsilateral and crossed pathways terminated at different dendritic levels (medial, $P = 0.2087$, $n = 5$, manipulation 3; lateral, $P = 0.8304$, $n = 9$, manipulation 4). Although LTD can be induced without prior induction of LTP, the first manipulation was omitted from this analysis in order to employ a repeated-measures analysis. The same results are obtained, however, if the values from the first manipulation are used in a nonrepeated-measures analysis (results not shown).

DISCUSSION

The present study indicates that in temporodentate pathways, the associative interactions between afferents that lead to LTP and LTD depend upon the degree of spatial overlap between the interaction pathways. In the present study, associative interactions were not observed when the degree of overlap between terminal fields, as revealed by the CSD analysis, was <50%. It is important to note that interactions can occur, however, when the extent of overlap is greater; in fact, there is a correlation between the degree of LTP or LTD elicited and the extent of overlap between interacting pathways (unpublished results). Interactions between spatially segregated pathways have also been observed under some conditions in the CA1 region of the hippocampus (19, 20).

The present results support the notion that the development of synaptic modification is under the control of at least two intracellular processes. One process is permissive for synaptic modification; this process is set into motion by a sufficient, perhaps even a threshold, level of depolarization in the dendrite of the postsynaptic cell. Such depolarization is normally produced by excitatory afferent activity. Our results indicate that the apparent threshold level of depolarization, as well as the intracellular permissive processes set into motion by sufficient depolarization, can be restricted to a portion of a dendrite. Thus, contrary to the hypothesis proposed by Hebb (7), it seems that the dendritic tree can function as more than one integrative unit during the induction of associative LTP and LTD. Within the region where the threshold for modification is surpassed, a second process governs the direction of modification (i.e., potentiation or depression) (21). This second process is determined entirely by activity at an individual synapse. If the synapse is active, potentiation is observed; if the synapse is silent, depression is observed.

It is important to distinguish between regulatory processes involved in LTP and LTD and the actual substrate of long-term, altered efficacy. The two processes considered above determine whether modification will take place and in which direction (potentiation or depression). Although the postsynaptic cell is the most likely site of these decision-making processes, the actual mechanisms of the altered efficacy could be presynaptic, postsynaptic, or both (22–27). In any event, it appears that LTP and LTD can be regulated by processes that occur within spatially restricted postsynaptic compartments.

Three hypotheses could account for the observed spatial properties. First, electronic decrement of depolarization could account for the amplitude, which is very near the threshold for LTP; even a 10–15% decrement in dendritic depolarization could result in subthreshold depolarization outside of the primary region of synaptic activation. Second, dendritic inhibition could account for the spatial property. This inhibition could substantially decrease dendritic depolarization outside the immediate area of activation (28). In this regard, it is interesting that one of the studies that demonstrates interactions between spatially segregated afferents in the CA1 region used picrotoxin-treated slices (20). Third, the spatial property could be accounted for if synaptic modification were dependent upon a biochemical event that remained localized to areas of strong synaptic activity.

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