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# Commissural synapses, but not mossy fiber synapses, in hippocampal field CA3 exhibit associative long-term potentiation and depression

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When CA3 commissural afferents received low-frequency (weak) stimuli synchronized with a train of mossy fiber bursts (strong), associative long-term potentiation (LTP) was induced at mixed commissural and associational synapses on hippocampal CA3 pyramidal cells in vitro. In contrast, a weak mossy fiber input did not potentiate when given in phase with commissural/associational bursts. Furthermore, commissural/associational synapses receiving low-frequency stimuli out-of-phase with strong rhythmic mossy fiber input showed associative long-term depression (LTD), whereas mossy fiber synaptic strengths were not depressed when they received weak inputs out-of-phase with a strong commissural/associational input. Thus, both associative LTP and associative LTD can be induced at commissural/associational synapses, but not at mossy fiber synapses.

Long-term potentiation (LTP), a long-lasting synaptic enhancement, is one of the most promising candidates for a cellular mechanism of learning and memory in the vertebrate brain[4]. LTP in the hippocampus is induced by brief, high-frequency stimulation of excitatory afferents and can last for many hours in vitro, and weeks or months in vivo[3]. Studies of hippocampal LTP indicate that its induction in the dentate gyrus and field CA1 requires simultaneous prolonged depolarization of the postsynaptic membrane and activation of glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype[10]. Commissural synapses terminating on CA3 pyramidal cells also exhibit a similar type of LTP, but recent evidence suggests that mechanisms underlying the induction of LTP at synapses of mossy fibers on CA3 pyramidal cells are fundamentally different. First, the mossy fiber terminal region is relatively lacking in NMDA receptors in comparison with other areas of the hippocampus[16]. Second, the induction of LTP at mossy fiber–CA3 synapses is not blocked by the NMDA antagonist, 2-amino-5-phosphonovalerate (AP5)[6]; however, at neighboring commissural synapses on the same neurons, AP5 reversibly blocks LTP[6,7].

LTP is a homosynaptic phenomenon specific to those synapses receiving high-frequency activation. A related heterosynaptic phenomenon, known as associative LTP, is elicited when weak excitatory inputs, which do not produce LTP when stimulated alone, are activated simultaneously with separate stronger inputs to the same neurons[2,11,12]. In addition, heterosynaptic long-term depression (LTD) has been reported in inputs silent during postsynaptic burst firing[1,13]. Recently, another type of LTD has been reported in field CA1 that can be induced by associating a weak and strong input on the same dendritic trees, but only if the inputs are anti-correlated[22]. Studies of LTP in field CA3 indicate that there may be differences in the associative interactions between commissural and mossy fiber

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inputs in field CA3[9]. We report here that both associative LTP and associative LTD can be induced in the commissural/associational inputs, but not in the mossy fiber inputs to CA3 pyramidal neurons.

Adult male Sprague–Dawley rats (100–200 g) were sacrificed under deep ether anesthesia. Hippocampal slices (400 $\mu$m thick) were prepared by conventional methods[15] and maintained at 34 °C, pH 7.4, at the interface between artificial cerebrospinal fluid (ACSF) and humidified 95% $O_2$/5% $CO_2$. ACSF composition was (mM): NaCl 126, KCl 5, $NaH_2PO_4$ 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. Stimuli were applied via bipolar stimulating electrodes of teflon-coated 20 $\mu$m diameter platinum–iridium wire. Extracellular field potential recordings were made in the CA3 pyramidal cell body layer and apical dendritic layer (Fig. 1A). Stimulating electrodes were placed on opposite sides of the hippocampal fissure, the first activating mossy fiber (MF) afferents and the second activating fibers arising from both commissural inputs and recurrent associational fibers originating from pyramidal cells in CA3 (COM/ASSOC) (Fig. 1A). Control experiments were performed to ensure that these stimulus sites activated two non-overlapping inputs (lack of cross-facilitation with paired pulse stimuli) that converge on a common population of CA3 pyramidal neurons (inputs did not summate when activated simultaneously). In some control experiments, specific activation of mossy fibers was verified by inducing LTP in the presence of AP5. The degree of potentiation or depression was evaluated by measuring the changes in amplitude of the population spike and the peak initial slope of the compound excitatory postsynaptic potential (EPSP). Results are expressed as mean percent change ± S.E.M.

The stimulus patterns used are shown in Fig. 1B. The strong stimulus pattern, which was effective in eliciting maximal LTP in over 90% of the slices, consisted of trains of 10 bursts of 5 pulses each at a frequency of 100 Hz, with a 200 ms inter-burst interval. Each train lasted 2 s and had a total of 50 stimuli. The weak stimuli, a train of single shocks at 5 Hz frequency, were given either superimposed on the middle of each burst (positively correlated, or IN PHASE), or symmetrically between the bursts (negatively correlated, or OUT OF PHASE). This pattern of stimuli was based on a neural network model of associative memory that predicts both long-term enhancement of synaptic strength when pairs of inputs are correlated, and reduction when they are anti-correlated[18,19]. Recent studies in our laboratory demonstrate an associative LTD of synaptic strength in field CA1 with out-of-phase stimuli[20,22]. The present study examines whether

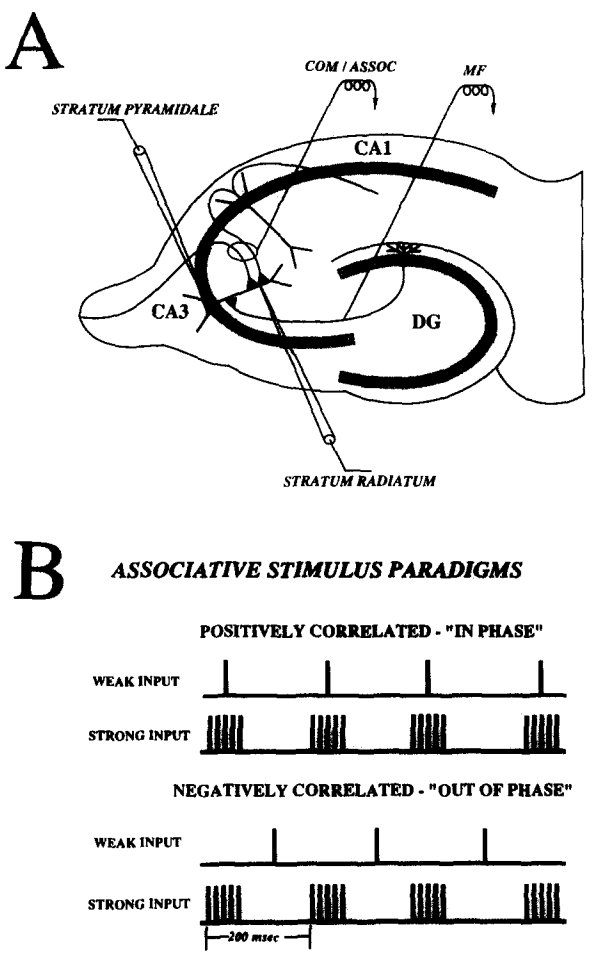


Fig. 1. Hippocampal slice preparation and stimulus paradigms. A: schematic diagram of the in vitro hippocampal slice preparation showing recording sites in the CA3 pyramidal cell somatic (STRATUM PYRAMIDALE) and apical dendritic (STRATUM RADIATUM) layers. Synaptic responses were elicited via stimulating electrodes in the stratum radiatum near CA2 to activate commissural/associational fibers (COM/ASSOC) and in the hilus of the dentate gyrus (DG) to activate the mossy fiber (MF) afferents. B: schematic diagram of stimulus paradigms. The strong input stimuli (STRONG INPUT) consisted of 4 sets of 2 s trains of 100 Hz bursts. Each burst had 5 pulses and the interburst interval was 200 ms. Weak input stimuli (WEAK INPUT), consisting of 4 sets of 2 s trains of single shocks at 5 Hz frequency, was either superimposed on the middle of each burst (IN PHASE), or placed symmetrically between the bursts (OUT OF PHASE).

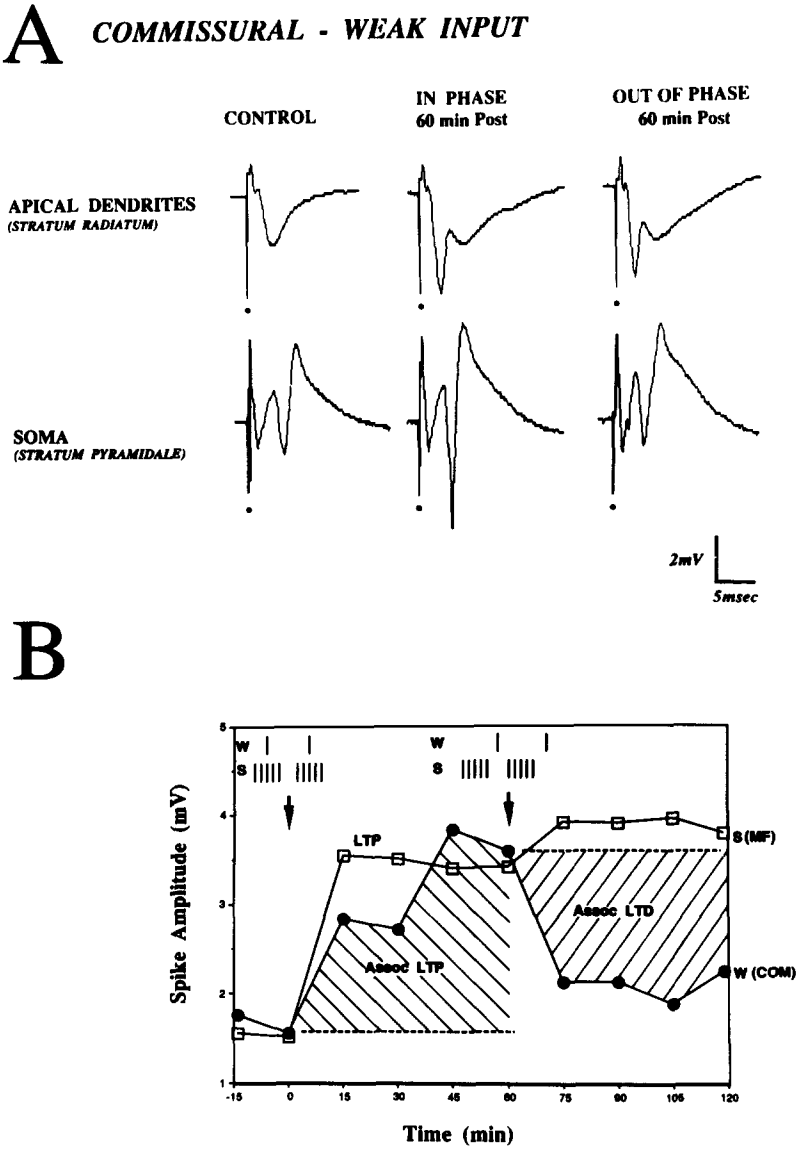


Fig. 2. CA3 commissural/associational synapses exhibit both associative LTP and LTD. A: when the COM side received a weak (W) input IN PHASE with a strong (S) MF tetanus, the W site exhibited associative long-term potentiation (LTP) of both the synaptic EPSP in the apical dendritic layer (upper traces) and the population action potential in the cell soma layer (lower traces). This was followed by application of W stimuli to the COM site OUT OF PHASE with the S stimuli to MF, which caused an associative long-term depression (LTD) seen as a marked reduction in the population spike amplitude with a lesser decrease in EPSP slope. Test responses are shown before (CONTROL) and 60 min after application of weak stimuli IN PHASE and OUT OF PHASE with the strong MF input, respectively. There was no significant change in the amplitude of the antidromic population spike following either in phase ($-0.73 \pm 1.7\%$, $n = 18$) or out of phase ($-4.04 \pm 2.2\%$, $n = 15$) stimulation. B: time course of the changes in population spike amplitude observed at each input for a typical experiment where synaptic strengths were alternately enhanced and depressed in the same slice. Test responses from the strong input (S, open squares), show that the high-frequency bursts elicited LTP specific to MF synapses. Test responses from the weak input (W, filled circles) show that stimulation of the weak pathway in phase with the strong one produced associative LTP (Assoc LTP) of this input. Associative LTD (Assoc LTD) of the same pathway was then elicited following out-of-phase stimulation.

similar forms of associative LTP and associative LTD are found in the commissural/associational and mossy fiber synapses onto CA3 pyramidal cells.

In initial experiments, the commissural/associational pathway was used as the weak input (W) site and the mossy fiber pathway received the strong stimuli (S). The weak stimulus train was first applied alone and did not itself induce long-lasting changes, fol-

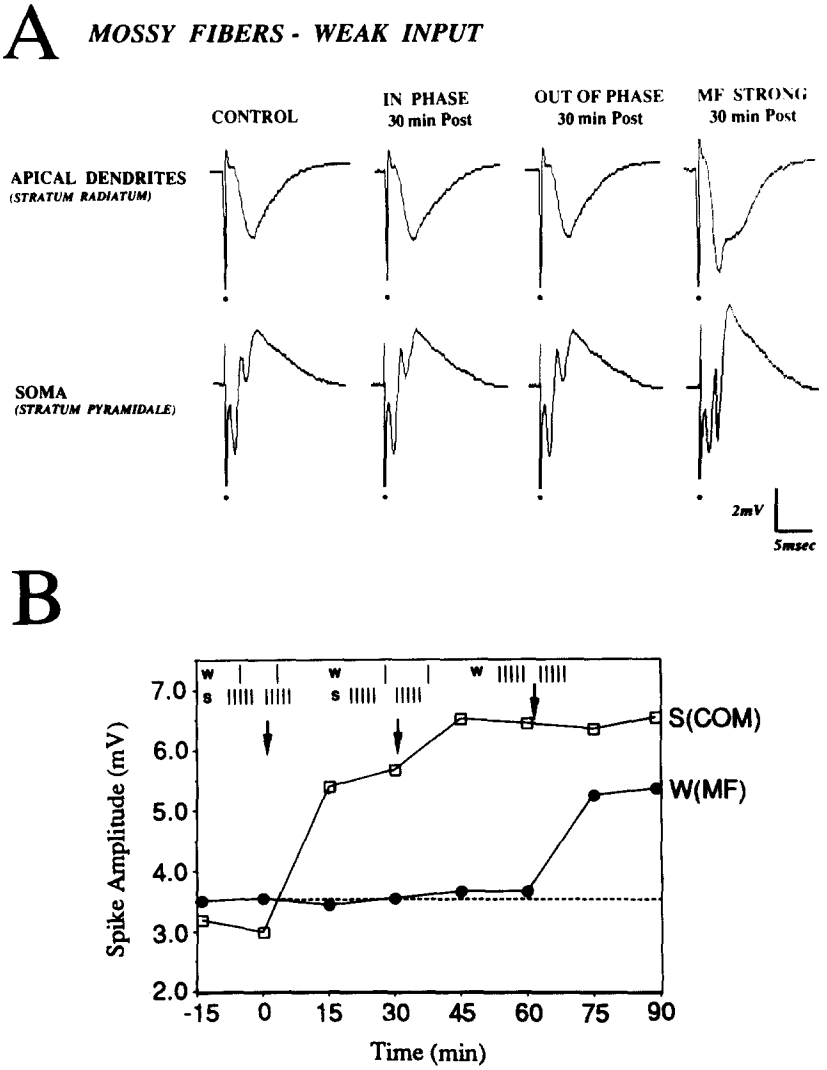


Fig. 3. CA3 mossy fiber synapses do not exhibit either associative LTP or LTD. A: when the MF pathway received weak (W) stimuli IN PHASE with strong (S) stimuli via the COM input, weak input MF synapses did not exhibit potentiation of either the synaptic EPSP in the apical dendritic layer or population spike in the cell body layer. Following this, application of W to the MF site OUT OF PHASE with S to COM, also failed to elicit depression in population spike or EPSP. Finally, MF synapses did show homosynaptic LTP when presented with an S tetanus (MF STRONG) to the MF pathway alone. Test responses are shown before (CONTROL) and 30 min after application of IN PHASE, OUT OF PHASE and MF STRONG stimuli, respectively. B: time course of the changes in population spike amplitude observed at each input for a representative experiment. Test responses from the strong input (S, open squares), show that the high-frequency bursts elicited homosynaptic LTP specific to COM synapses. Test responses from the weak input (W, filled circles) show that neither IN PHASE nor OUT OF PHASE stimulation elicited any potentiation or depression of this input. However, MF synapses did show homosynaptic LTP following the application of the strong bursts (S) to the MF pathway alone.

lowing which strong site stimulation alone elicited homosynaptic LTP of the strong pathway. The strong site stimulation by itself, did not significantly alter either the synaptic EPSP (+5.45 ± 1.1%, $n$ = 19) or population action potential (+3.92 ± 3.5%, $n$ = 19) of the weak input. However, when the commissural/associational (COM) side received a weak input in phase with a strong mossy fiber (MF) tetanus, it elicited an associative LTP of the weak input synapses, as shown in Fig. 2A. Both the synaptic EPSP (Fig. 2A, upper traces; Fig. 4, COM=W site, hatched bar) and population action potential (Fig. 2A, lower traces; Fig. 4, COM=W site, solid bar) were significantly enhanced for at

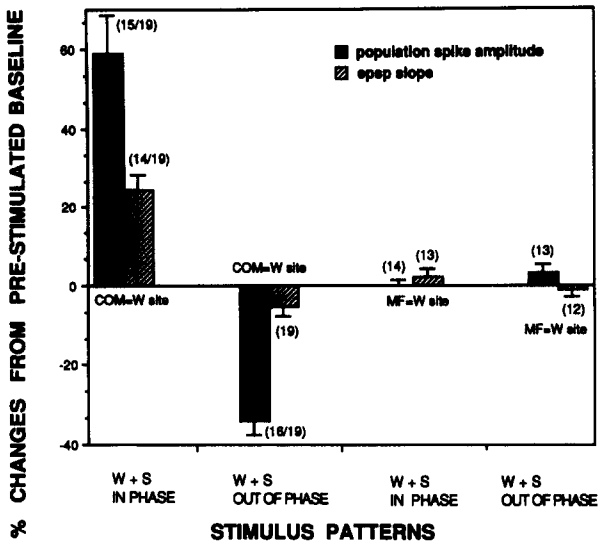


Fig. 4. Mean associative LTP and LTD in field CA3. At weak input COM synapses, IN PHASE stimulation elicited associative LTP of both population spike amplitude (solid bar) and EPSP slope (hatched bar), while OUT OF PHASE stimulation caused associative LTD of population spike amplitude with a lesser decrease in EPSP slope. All values are mean ± S.E.M. percent change relative to control pre-stimulated baselines and numbers in parentheses adjacent to each bar indicate [number of slices showing statistically significant associative LTP or LTD]/[total number of slices] ($P < 0.01$, $t$-test) or (total number of slices) where no changes were observed.

least 60 min, up to 180 min, following stimulation.

In contrast, application of weak stimuli to the commissural/associational site out-of-phase with the strong mossy fiber tetanus caused an associative long-term depression (LTD) of the weak input synapses (Fig. 2A; Fig. 4). There was a marked reduction in the population spike (Fig. 4) with smaller decreases in the EPSP. In 8 of the 19 slices there was a marked decrease (–15.4 ± 3.0%, $n = 8$, $P < 0.01$, $t$-test) in EPSP and the remaining 11 showed no significant decrease. Note that the stimulus patterns applied to each input were identical in these two experiments, and only the relative timing of the weak and strong stimuli was varied. Synaptic strengths could be alternately enhanced and depressed in the same slice (Fig. 2B), independently of the sequence in which the in phase and out-of-phase stimulus patterns were presented.

In the second series of experiments, the stimulus paradigms were reversed so that the mossy fibers were the weak input and the strong tetanus was applied to commissural/associational afferents. The application of weak stimulus alone did not itself induce long-lasting changes and the strong-site stimulation alone elicited homosynaptic LTP of the strong pathway without significantly altering weak input synaptic EPSP (+1.0 ± 1.1%, $n = 14$) or population spike (+2.56 ± 1.4%, $n = 14$). In contrast to commissural/associational synapses, when the mossy fiber pathway received weak stimuli in phase with strong stimuli via the commissural/associational input, mossy fiber synapses did not exhibit potentiation of either synaptic EPSP (Fig. 3A; Fig. 4, MF=W site, hatched bar) or population spike (Fig. 3A; Fig. 4, MF=W site, solid bar). Similarly, application of weak stimuli out-of-phase with strong stimuli also failed to elicit LTD of the EPSP or population spike (Fig. 3; Fig. 4, MF=W site, W+S OUT OF PHASE). Furthermore, the weak input mossy fiber synapses failed to exhibit associative LTP or LTD, irrespective of the order in which the stimulus patterns were presented. Finally, although a weak input to mossy fiber synapses failed to elicit either associative LTP or LTD, it did show homosynaptic LTP in response to a strong tetanus to mossy fiber afferents alone (Fig. 3, MF STRONG). After induction of mossy fiber LTP in 4 slices, the mossy fiber synaptic strength was not altered when this homosynaptically potentiated mossy fiber pathway received weak stimuli out-of-phase with strong commissural/associational stimulation.

Our studies of interactions between strong and weak inputs onto the same dendritic tree of hippocampal pyramidal cells of field CA3 suggest that these pyramidal cells receive two separate synaptic inputs that differ fundamentally. The CA3 commissural/associational synapses, which depend on NMDA receptor activation for the induction of LTP, exhibit associative LTP when they receive weak stimuli positively correlated in time with a strong mossy fiber tetanus. This result supports the existing evidence that these synapses show depolarization-dependent associativity, a property thought to derive from the voltage dependence of the NMDA receptor[5,10,14,17]. The CA3 commissural/associational synapses are also interesting from a computational perspective because of our finding that when the same weak input is negatively correlated in time with the strong mossy fiber input, a long-term depression (LTD) of the weak commissural/associational input is induced.

The rules for induction of long-term synaptic plasticity in mossy fiber inputs onto CA3 pyramidal

neurons is fundamentally different from those of commissural/associational inputs. We have shown that a weak mossy fiber input failed to exhibit associative LTP when it was positively correlated in time with a strong commissural/associational tetanus. This finding supports previous evidence[9] and can be explained by the lack of NMDA receptors at this synapse[16]. The failure of these synapses to elicit associative LTD, however, is more surprising, since results from similar experiments in field CA1 suggest that postsynaptic hyperpolarization coupled with presynaptic activation triggers associative LTD without requiring NMDA receptor activation[22]. Our findings do not, however, rule out long-lasting potentiation or depression at these synapses that may require different stimulus patterns or important modulatory factors supplied by subcortical afferents to field CA3[8,21].

The CA3 commissural/associational input exhibits a novel form of synaptic plasticity which is homosynaptic and follows a Hebbian covariance rule similar to that found in field CA1[20,22]. Taken together, associative LTP and LTD may permit storage at commissural/associational synapses of covariance information between commissural/associational and mossy fiber inputs[18,19]. The striking differences between synapses onto CA3 pyramidal neurons are likely to reflect their different roles in processing the flow of information from dentate granule cells to CA1 pyramidal neurons. The mossy fibers seem to be providing a 'teaching' signal that 'instructs' the commissural/associational inputs through associative interactions, since they can influence, but cannot be influenced by, signals arriving from other pathways.

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