Neuron, Vol. 18, 295–305, February, 1997, Copyright ©1997 by Cell Press

Hippocampal Interneurons Express a Novel Form of Synaptic Plasticity

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Summary

Individual GABAergic interneurons in hippocampus can powerfully inhibit more than a thousand excitatory pyramidal neurons. Therefore, control of interneuron excitability provides control over hippocampal networks. We have identified a novel mechanism in hippocampus that weakens excitatory synapses onto GABAergic interneurons. Following stimulation that elicits long-term potentiation at neighboring synapses onto excitatory cells, excitatory synapses onto inhibitory interneurons undergo a long-term synaptic depression (interneuron LTD; iLTD). Unlike most other forms of hippocampal synaptic plasticity, iLTD is not synapse specific: stimulation of an afferent pathway triggers depression not only of activated synapses but also of inactive excitatory synapses onto the same interneuron. These results suggest that high frequency afferent activity increases hippocampal excitability through a dual mechanism, simultaneously potentiating synapses onto excitatory neurons and depressing synapses onto inhibitory neurons.

Introduction

The hippocampus contains a relatively small number of inhibitory interneurons, which control the output of large numbers of excitatory pyramidal neurons (Schwartzkroin and Mathers, 1978; Knowles and Schwartzkroin, 1981; Kawaguchi and Hama, 1987; 1988; Lacaille et al., 1987; 1989; Lacaille and Schwartzkroin, 1988a; 1988b; Sik et al., 1995). Interneurons have extensive axonal arbors and innervate pyramidal cells multiple times, suggesting that each time an interneuron fires an action potential, hundreds of postsynaptic cells will be reliably and simultaneously inhibited (Schwartzkroin and Kunkel, 1985; Li et al., 1992; Buhl et al., 1994a; Cobb et al., 1995; Sik et al., 1995). Normal brain function in the cortex and hippocampus depends critically on the inhibitory drive contributed by interneurons; for example, blockade of inhibition (via $GABA_A$ receptors) results in epileptiform activity (Wong and Prince, 1979; Wong and Traub, 1983; Sloviter, 1991). Damage to hippocampal interneurons may be involved in the generation and maintenance of epilepsy, in which hippocampal excitatory neurons become pathologically active and fire at high frequency (Sloviter, 1987; 1991; 1992; Stelzer et al., 1987; Scharfman and Schwartzkroin, 1990; Zhao and Leung, 1991).

Surprisingly little is known about the control of interneuron excitability; most analyses of inhibitory function have focused on the role of GABAergic inhibition studied indirectly in excitatory neurons. Moreover, although considerable emphasis has been placed on plasticity of excitatory synapses onto excitatory neurons, the equally important role of inhibitory interneurons in modifying the behavior of hippocampal circuitry has received little attention.

We have examined synaptic plasticity of stratum radiatum (s. radiatum) interneurons in the hippocampal CA1 region, which is the site of the most intensively studied form of long-term potentiation (LTP). Excitatory afferents from the CA3 region make en passant synapses on multiple pyramidal cells that exhibit synapse-specific (homosynaptic) LTP. The same CA3 afferents excite not only pyramidal cells but also their interneuron neighbors in s. radiatum, which probably mediate the powerful "feedforward" inhibition present in CA1 pyramidal cells (Fox and Ranck, 1981; Alger and Nicoll, 1982; Buzsaki and Eidelberg, 1982; Buzsaki, 1984; Ashwood et al., 1984; Frotscher et al., 1984; Schwartzkroin and Kunkel, 1985).

Using visualized whole-cell patch-clamp recordings, we find that the same stimulation that elicits LTP in pyramidal cells markedly depresses excitatory synapses onto CA1 interneurons in s. radiatum. We further report that, unlike most other forms of synaptic plasticity in hippocampus, the long-lasting depression in interneurons weakens not just active synapses but multiple excitatory synapses onto the same interneuron. An obvious consequence of this depression will be a potent enhancement of the excitability of hundreds of pyramidal cells normally innervated by each interneuron.

Results

Basic information regarding the physiological properties of interneurons is limited compared with what is known about excitatory pyramidal neurons. This situation arose because interneurons are sparse and widely scattered, making them unlikely targets for an electrode aimed blindly at the brain slice (Figure 1A), and because interneurons are notoriously difficult to record from for long periods using sharp microelectrodes. We have successfully used visualized whole-cell patch-clamp recordings to overcome these problems (Sah et al., 1996).

To appreciate the functional role of s. radiatum interneurons, the local circuit in which they participate must be defined. These interneurons receive excitatory afferent input from CA3 pyramidal cells and provide feedforward inhibition to hundreds of CA1 pyramidal cells (Figure 1B). The CA1 pyramids, in turn, send recurrent collaterals to innervate interneurons. The peak of the EPSC recorded from an s. radiatum interneuron precedes the peak of the population pyramidal cell excitatory postsynaptic potential (EPSP) (Figure 1C), suggesting that these interneurons are indeed activated before CA1 pyramidal cells and provide feedforward inhibition (latency from stimulus to field potential onset, mean $\pm$ SEM: 3.9 $\pm$ 0.2 ms; latency to onset of interneuron EPSC/EPSP: 2.8 $\pm$ 0.2 ms; $n = 23$; $p < .003$ by Student's *t* test). In the experiments below, we have focused exclusively on monosynaptic excitatory responses elicited by stimulation of CA3 afferents onto interneurons.

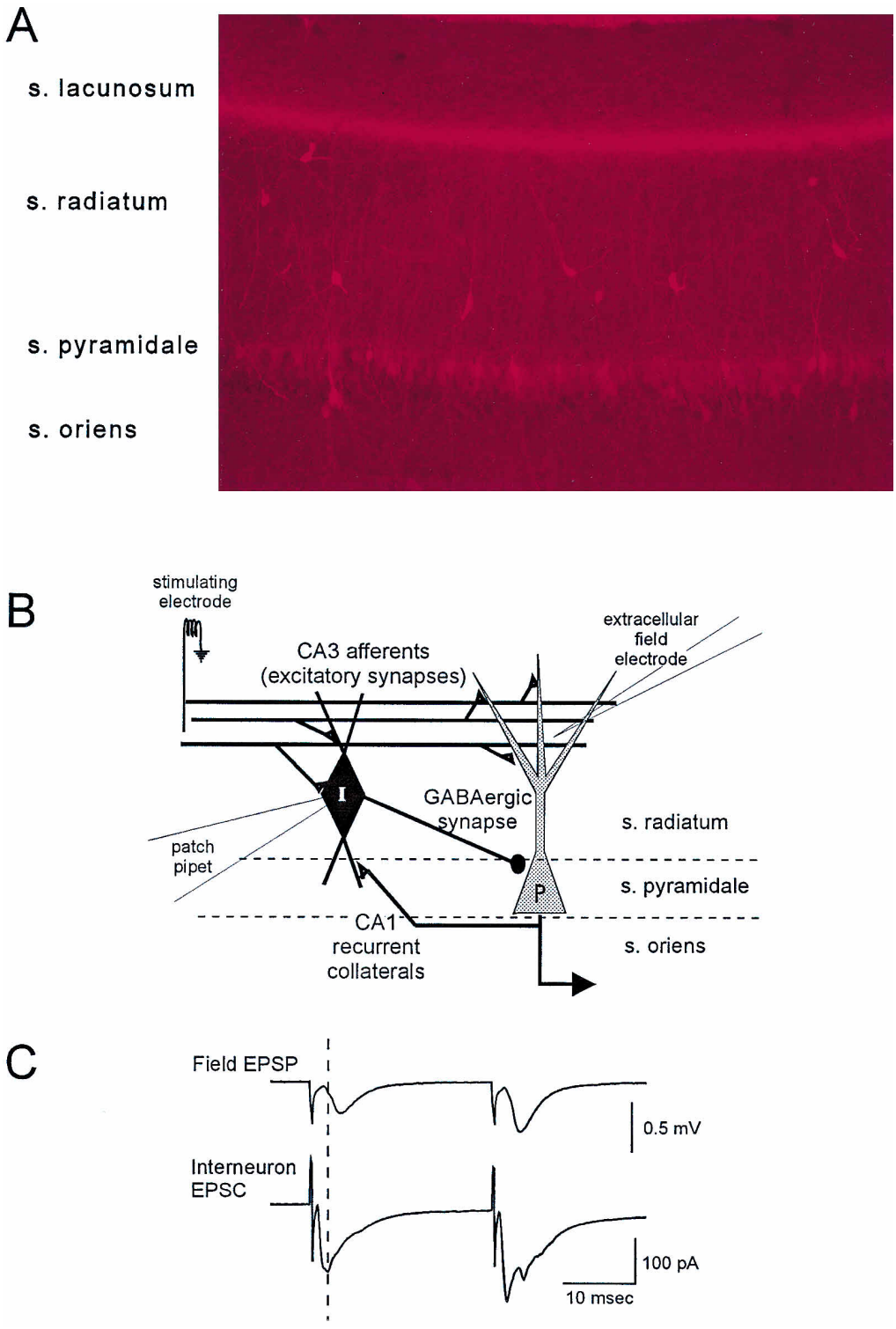

Figure 1. (A) Distribution of interneurons in s. radiatum. The majority of s. radiatum interneurons express the calcium-binding protein, calbindin (Freund et al., 1990; Toth and Freund, 1992); an anti-calbindin antibody was used to visualize the distribution of s. radiatum interneurons within the slice preparation (1 cm = 100 μm).

(B) Diagram of an s. radiatum interneuron in the local circuit in area CA1. Glutamatergic afferents from CA3 pyramidal cells (Schaffer collaterals) synapse both onto the s. radiatum interneuron and onto dendrites of CA1 pyramidal cells. Stimulation of this pathway excites both cell types. The GABAergic interneuron forms synapses onto pyramidal neurons, at either primarily somatic or primarily dendritic regions (Figure 5). CA1 pyramidal cells extend local excitatory collaterals that innervate interneurons.

(C) Stimulation of CA3 afferents elicits a field EPSP in CA1 pyramidal cells and a simultaneously recorded EPSC in a voltage-clamped interneuron. The peak of the interneuron EPSC precedes the peak of the field EPSP, demonstrating that these interneurons are positioned to participate in feedforward inhibition. The traces represent the average of 10 EPSCs and EPSPs.

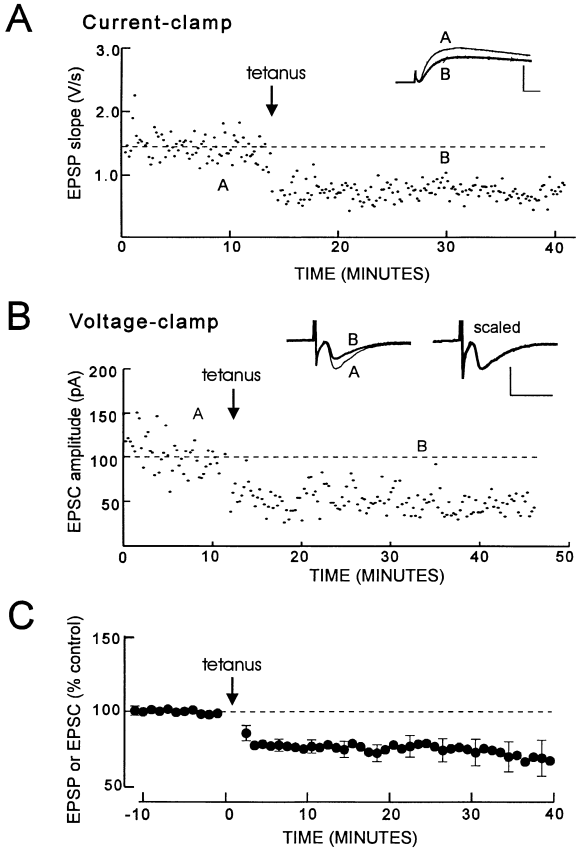

Figure 2. iLTD Is Induced After High Frequency Stimulation of CA3 Afferents

(A) Current-clamp recording from an interneuron before and after 100 Hz tetanus (arrow). Example traces (inset) are taken from portions of the time course marked A (control) and B (after tetanus). In this example and others, each point represents the average of three EPSPs. Calibration: 10 mV, 10 ms.

(B) Voltage-clamp recording from an interneuron before and after 100 Hz tetanus (arrow). Example traces (inset) are taken at the times marked A (control) and B (after tetanus). The right-hand inset shows the "after tetanus" EPSC scaled to match control amplitude. Calibration: 100 pA, 10 ms.

(C) Summary data from 49 interneurons receiving tetanus at the arrow. Experiments recording from interneurons in voltage clamp ($n = 20$) and current clamp ($n = 29$) are pooled in this figure. All experiments are included regardless of whether iLTD was observed. Error bars in this figure and all others represent the mean $\pm$ SEM.

High Frequency Stimulation Elicits LTD in Interneurons

Brief high frequency stimulation of CA3 afferents triggers LTP at excitatory synapses onto CA1 pyramidal cells (Andersen et al., 1973; Alger and Teyler, 1976). We began by examining the effects of stimulating the same afferents onto neighboring interneuron synapses, specifically asking if they are enhanced like synapses onto pyramidal cells. We therefore recorded EPSPs in s. radiatum interneurons before and after tetanic stimulation of presynaptic Schaffer collateral axons (100 Hz for 1 s). In sharp contrast to the synaptic potentiation that this stimulation evokes in pyramidal cells, EPSPs in interneurons were markedly reduced in amplitude immediately after tetanic stimuli (Figure 2A). In theory, changes in voltage-dependent conductances in the postsynaptic cell could account for the apparent synaptic depression observed in current-clamp recordings. However, EPSC depression was also observed in experiments using voltage-clamp conditions, demonstrating that voltage-dependent conductances do not contribute significantly to the synaptic depression (Figure 2B).

In experiments from 49 interneurons, excitatory neurotransmission in the majority was depressed after tetanus (Figure 2C). EPSP/Cs were depressed in 32 cells, unchanged in 14 cells, and slightly potentiated in 3 cells. The depression occurred within 5 s after tetanus, and posttetanic potentiation was generally not observed. In 9 of 32 cells, the EPSP/C returned to baseline levels within 20 min. However, in the majority of interneurons (23/32), synaptic depression persisted for the duration of the recording (up to 70 min after tetanus). We refer to this form of synaptic depression as "interneuron long-term depression" (iLTD).

Synapses onto Interneurons Undergo LTD When Pyramidal Cell Synapses Undergo LTP

Thus, the same stimulus paradigm that produces LTP in pyramidal cells produces iLTD in interneurons. To demonstrate clearly that a 100 Hz tetanus triggers simultaneous LTP in CA1 pyramidal cells and LTD in interneurons, we recorded from both cell types simultaneously. We delivered a tetanus to CA3 afferents and recorded both from an interneuron, using a patch electrode, and from local pyramidal cells, using an extracellular recording electrode (Figure 3A). While the tetanus triggered LTP in the surrounding pyramidal cells, EPSP/Cs in the majority of interneurons were again depressed (13 of 23 cells) (Figure 3B). Thus, in contrast to the simple expectation that all synapses of CA3 neurons will express LTP after tetanus, the same stimulus paradigm that reliably produces LTP in pyramidal cells produces iLTD in interneurons.

A second stimulus paradigm that is very effective at triggering pyramidal cell LTP is known as "pairing": low frequency stimulation of afferent fibers is paired with postsynaptic depolarization to unblock NMDA receptors and produce LTP (Kelso et al., 1986; Gustafsson et al., 1987; Kauer et al., 1988). To further explore the nature of iLTD, we used the pairing protocol with interneurons. Depolarization of interneurons "paired" with 60 presynaptic stimuli at 1 Hz failed to change the strength of excitatory inputs onto interneurons (Figure 3C), suggesting that the requirements to initiate pyramidal cell LTP differ from those required to initiate iLTD (EPSP slope 5 min postpairing: 103% $\pm$ 5% of control; $n = 13$). Subsequent tetanic stimulation triggered iLTD (EPSP slope 5 min posttetanus: 85% $\pm$ 8%; $n = 9$). In support of the idea that iLTD is distinct from LTP, iLTD was still observed even when voltage-clamped interneurons received tetanus without concomitant postsynaptic depolarization ($n = 3$), a condition that reduces or blocks LTP induction in pyramidal cells (Malinow and Miller, 1986; Kelso et al., 1986).

Interneuron LTD Is Not Synapse Specific

Excitatory synapses onto pyramidal cells undergo LTP in response to tetanic stimulation, but after prolonged

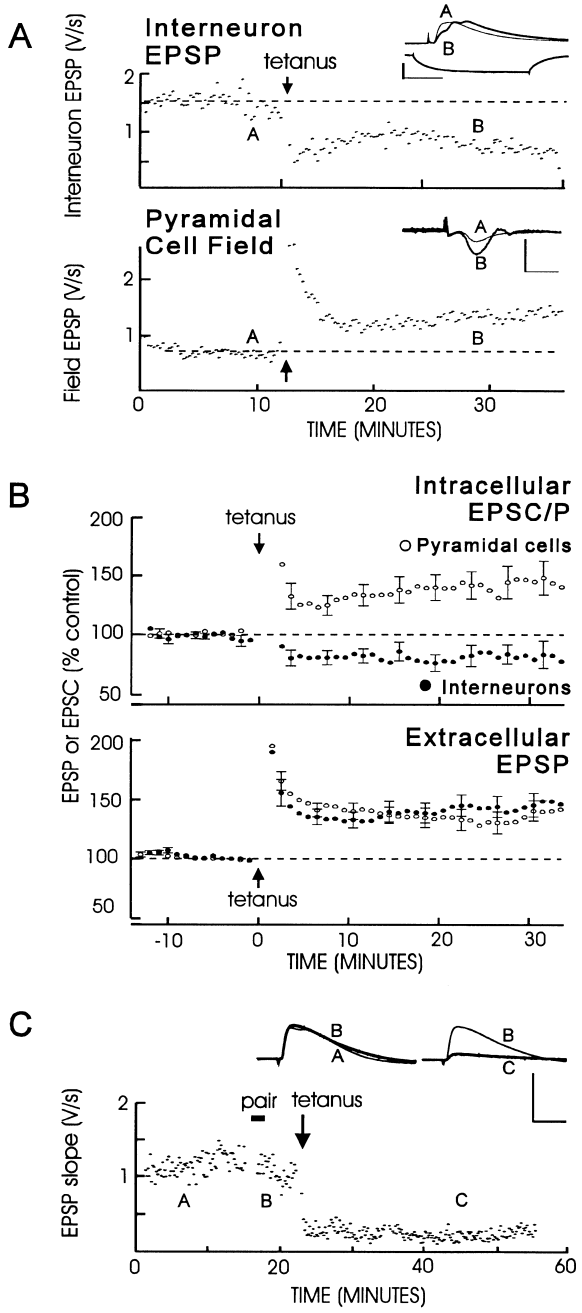

Figure 3. Tetanus Simultaneously Elicits iLTD in Interneurons and LTP in CA1 Pyramidal Cells

(A) Typical experiment illustrating that an interneuron recorded under current clamp shows synaptic depression immediately after tetanus (top graph), while neighboring CA1 pyramidal cells respond to the same tetanus with LTP (bottom graph). Top inset shows the interneuron EPSP and response to a hyperpolarizing current step before and 20 min after tetanus. The EPSP is depressed while the cell input resistance is unchanged. Bottom inset shows extracellular field EPSPs recorded at the same time points. Calibration: top graph, 15 mV, 20 ms; bottom graph, 0.5 mV, 10 ms.

(B) Summary graph of experiments recording either simultaneous intra- and extracellular recordings from pyramidal cells (open circles, n = 13) or simultaneous intracellular recordings from interneurons and extracellular recordings from pyramidal cells (closed circles, n = 23). Tetanus was delivered at the arrows. The top graph compares intracellularly recorded pyramidal cell potentiation with lower frequency stimulation (e.g., 1 Hz) also express LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992). LTP and LTD in pyramidal cells are homosynaptic: only activated synapses are enhanced or depressed, while inactive synapses remain unaffected (Andersen et al., 1977; 1980; Lynch et al., 1977; Dudek and Bear, 1992; Mulkey and Malenka, 1992).

Because synaptic plasticity in pyramidal cells is homosynaptic, we were interested in determining whether iLTD is similar. To test this idea directly, we recorded from an interneuron while alternately stimulating two independent excitatory synaptic inputs onto the cell. Tetanic stimulation was delivered to only one of the two afferent pathways, while the result was monitored in both (Figure 4A and 4B). After tetanus to one input, EPSCs in both pathways were immediately depressed. Similar results were observed in eight interneurons (Figure 4C). This result strongly argues that high frequency stimulation of one set of synapses depresses other, unstimulated excitatory synapses on the same interneuron.

Consistent with this idea, the late EPSP/C, which clearly arises from synapses that differ from those receiving tetanic stimulation (most likely from recurrent collaterals of CA1 neurons, see Figure 4D), was often depressed along with the monosynaptic EPSP/C. Since the CA3–CA1 pyramidal cell synapse is potentiated after tetanus, we expected the late recurrent EPSP/C in the interneurons to reflect this by potentiating as well (Maccaferri and McBain, 1995). Although this was observed in some cases (10 of 23 cells with an obvious late EPSP/C) (Figure 4D, bottom), in other interneurons, late polysynaptic events were depressed (13 of 23 cells) (Figure 4D, top). These data support the idea that iLTD is not synapse specific, triggering depression at multiple excitatory synapses onto the interneuron following high frequency stimulation of a single set of synapses. Thus, iLTD differs fundamentally from pyramidal cell LTP and LTD.

Interneurons That Undergo iLTD Do Not Form a Homogeneous Anatomical Class

Hippocampal interneurons are more heterogeneous than their pyramidal cell neighbors, varying in axon terminal fields, dendritic arrangement, and expression of neuropeptides and Ca^{2+}-binding proteins (Lorente de No, 1934; Schwartzkroin and Kunkel, 1985; Sloviter and Nilaver, 1987; Babb et al., 1988; Freund et al., 1990; Buhl et al., 1994b; McBain et al., 1994; Sik et al., 1995). To predict the effects of iLTD on local circuitry more thoroughly, each interneuron from which we recorded was

interneuron depression, while the bottom graph demonstrates that in both sets of experiments, field EPSPs were equally potentiated. (C) Pairing synaptic stimulation with postsynaptic depolarization does not induce iLTD. An interneuron was depolarized to −10 mV and stimulated 60 times at 1 Hz. When the membrane was repolarized, synaptic depression was not observed. Subsequent tetanic stimulation, however, triggered iLTD. Example traces (inset) illustrate EPSPs taken at the times denoted by A (control), B (after pairing), and C (20 min after tetanus). Calibration: 20 mV, 20 ms.

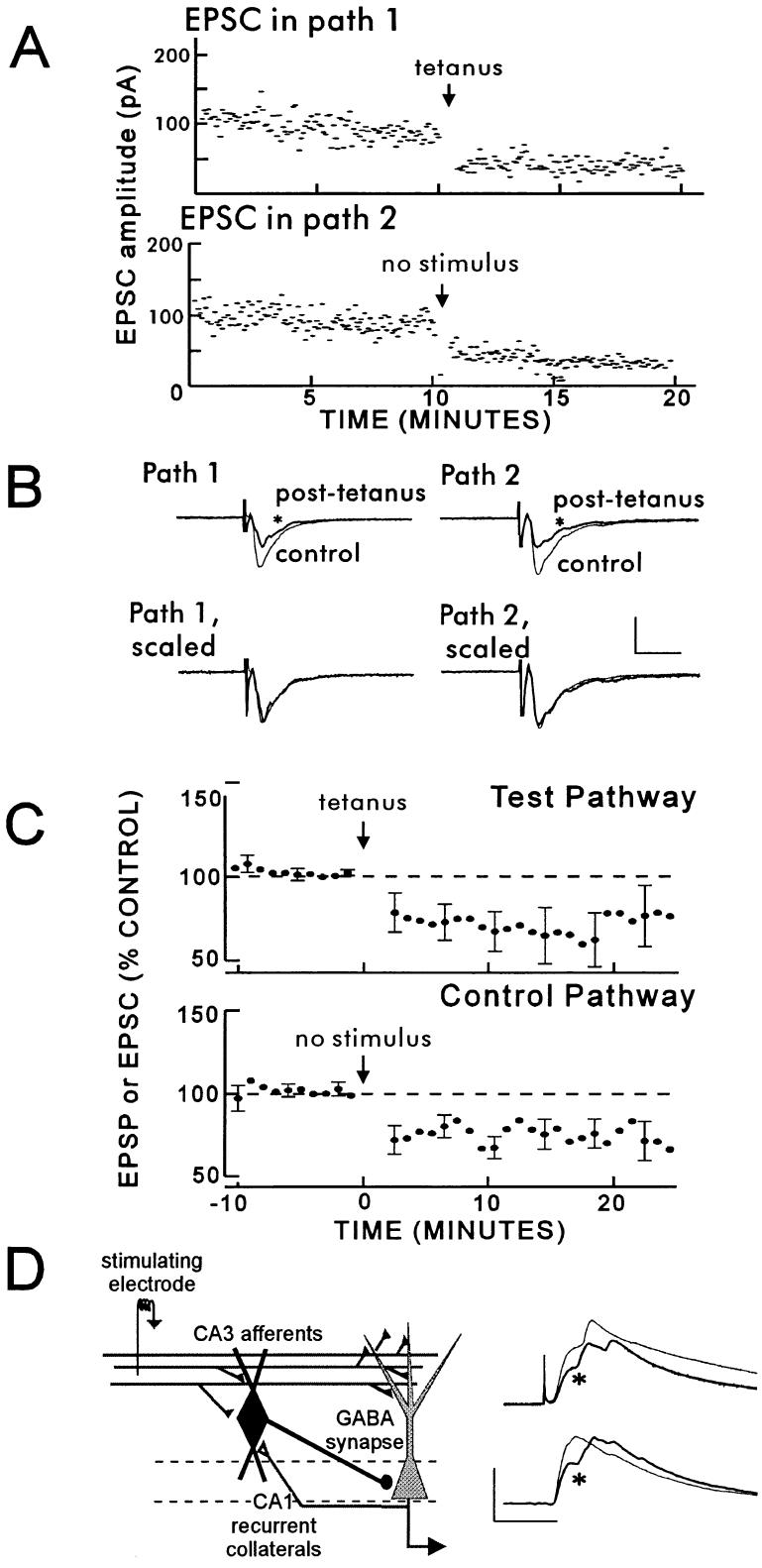

Figure 4. iLTD Is Not Synapse Specific

(A) Example of iLTD elicited by tetanus to a single afferent pathway but influencing EPSCs in a second, independent pathway recorded in an interneuron. Path 1 received tetanic stimulation at the arrow and exhibited LTD; path 2 was not stimulated at the arrow yet showed clear iLTD after tetanus to path 1.

(B) EPSCs taken from the experiment in (A). The top traces illustrate the iLTD observed in responses from path 1 (left) and path 2 (right). In the bottom traces, the depressed EPSC has been scaled to match the control amplitude. Calibration: 200 pA, 10 ms.

(C) Summary graph of eight experiments in which one of two independent pathways received tetanic stimulation. The top graph shows the mean responses in the pathway receiving tetanus, and the bottom graph shows the mean responses in the control, unstimulated pathway. Of these experiments, four were carried out under voltage-clamp and four under current-clamp conditions.

(D) Right lane: examples of EPSPs from two different interneurons. The initial EPSP reflects the monosynaptic input from CA3, while the late EPSPs may result from activation of recurrent collaterals from CA1 pyramidal cells (see circuit diagram). In each pair of traces, the thin line represents control EPSPs while the thick line represents EPSPs following tetanus. Although the monosynaptic EPSP is depressed in each of these cells, the late EPSP in one is depressed (top traces), while in the other it is enhanced (bottom traces). Calibration: 20 mV, 20 ms.

filled with biocytin, fixed, and reconstructed. Of 49 interneurons, 30 filled well enough to identify axon termination fields. Dendrites branched only sparsely and were aspiny. Cells typically had one of two distinct patterns of axonal arbor: either heavy innervation close to and within s. pyramidale, (basket cells, right-hand cell in Figure 5) or axonal branching throughout s. radiatum and s. oriens (bistratified cells, left-hand cell in Figure 5). The basket cells are likely to inhibit action potential generation at the cell body, while the bistratified cells

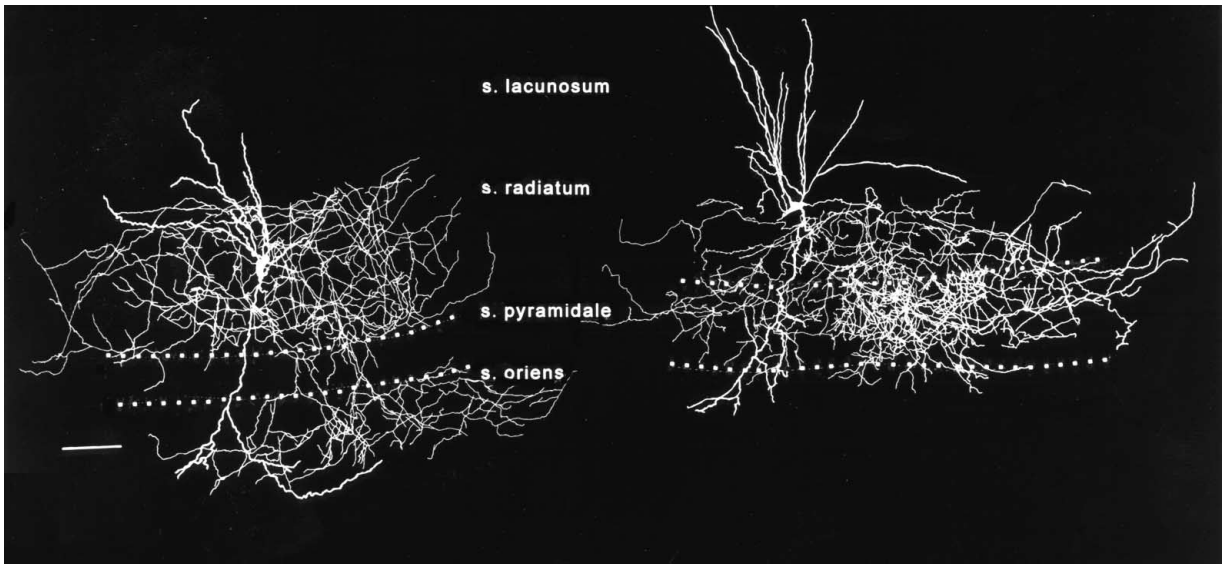

Figure 5. Morphological Features of s. radiatum Interneurons

Camera lucida drawings of the two major classes of interneurons in s. radiatum, as defined by axonal projections. Left: bistratified cell, with axon throughout s. radiatum and s. oriens. Right: basket cell, with axon primarily in s. pyramidale. Scale bar = 100 μm.

appear specialized to deliver inhibition to the dendrites, interacting there with excitatory synapses. The axon fields always extended for >500 μm parallel to s. pyramidale and left the plane of each 400 μm thick slice at both edges, suggesting that our measurements underestimate the true termination zone. These extensive axon arborizations support the idea that a single interneuron simultaneously inhibits hundreds of pyramidal cells, functionally grouping and synchronizing local excitatory cells.

Of 49 s. radiatum interneurons, 32 (65%) responded to tetanus with LTD. We hypothesized that perhaps one functional type of interneuron might express iLTD while the other might not. However, the ability to undergo iLTD is not correlated with the two morphologically identified cell types present in our material, based on axonal arborization: of 19 cells with identifiable axons that exhibited synaptic depression, 8 were bistratified cells, with axons in s. radiatum/s. oriens, while 11 were basket cells, with axons primarily innervating s. pyramidale. Therefore, each of the major interneuron types in s. radiatum can undergo synaptic depression after high frequency stimulation.

Discussion

High frequency stimulation of excitatory CA3 afferents results in a persistent depression of excitatory synapses onto interneurons. iLTD is triggered by the same stimulus pattern that causes neighboring excitatory synapses onto pyramidal cells to undergo LTP. Unlike LTP or LTD in excitatory hippocampal cells, iLTD is not synapse specific, as both mono- and polysynaptic excitatory inputs onto interneurons are depressed after tetanus, and tetanic stimulation delivered to one set of afferents produces concomitant depression in an independent set of afferents onto the same interneuron. Interneurons of both major anatomical classes (based on axonal morphology) exhibit iLTD.

Interneuron LTD May Explain Previous Observations

Indirect evidence from previous studies supports the idea that inhibitory circuits are depressed following stimulation that triggers LTP in pyramidal cells. In area CA3, following 20 Hz stimulation of excitatory afferents, previously unseen synaptic connections between pyramidal cells become apparent (Miles and Wong, 1987). This observation was attributed to reduced GABAergic inhibition that revealed "latent" excitatory connections. This result can be explained if significant iLTD follows 20 Hz stimulation.

A large body of literature has reported a form of enhancement termed EPSP–spike potentiation (E–S potentiation), both in area CA1 and in the dentate gyrus (Bliss and Lomo, 1973; Andersen et al., 1980; Wilson, 1981; Wilson et al., 1981; Abraham et al., 1985; 1987; Taube and Schwartzkroin, 1988; Chavez-Noriega et al., 1990; Tomasulo et al., 1991). High frequency stimulation not only triggers synaptic potentiation in pyramidal cells (LTP) but also reduces the threshold for action potential generation, such that an EPSP of a given size produces action potentials more easily. As $GABA_A$ antagonists strongly reduce E–S potentiation, at least part of the increased excitability results from disinhibition (Abraham et al., 1987; Tomasulo et al., 1991; but see Aszetely and Gustafsson, 1994). This finding suggests that excitatory synapses onto interneurons either fail to potentiate or are depressed following tetanic stimulation. iLTD almost certainly contributes to E–S potentiation, since following a tetanus, an excitatory input onto an interneuron will produce a smaller EPSC and a smaller downstream release of GABA onto $GABA_A$ receptors that normally control spike threshold in local pyramidal cells.

Synaptic Plasticity at Excitatory Synapses on Other Hippocampal Interneurons

Synaptic plasticity has been examined in other interneurons of the hippocampus. Following high frequency stimulation, excitatory responses in some interneurons have been reported to potentiate, some to depress, and some to remain unchanged (Buzsaki and Eidelberg, 1982; Taube and Schwartzkroin, 1987; Stelzer et al., 1994). In these studies, the recorded cells were not identified morphologically, making it difficult to correlate synaptic plasticity with interneuron type or function. More recently, a pairing protocol was reported to induce LTP in one class of s. radiatum nonpyramidal cell that was morphologically distinct from the s. radiatum interneurons in which we have observed iLTD; consistent with our observations, interneurons with morphologies like our cells always failed to exhibit LTP (Maccaferri and McBain, 1996). LTP at interneurons in a different stratum of CA1 (s. oriens) has been reported following tetanus (Ouardouz and Lacaille, 1995). It appears, however, that the potentiated synapse is on pyramidal cells, which in turn provide a larger recurrent EPSC to the interneuron; the monosynaptic EPSC from CA3 neurons does not support LTP (Maccaferri and McBain, 1995; 1996). Taken together, these experiments support the idea that the majority of excitatory synapses on hippocampal interneurons do not exhibit LTP.

The latter three studies were carried out at room temperature; one reason for the apparent absence of iLTD may be the temperature sensitivity of the phenomenon. In pilot studies for the present work, we found little or no depression following tetanic stimulation when experiments were carried out at 22°C (L. L. M. and J. A. K., 1995, Soc. Neurosci., abstract *20*, p. 847). Increasing the temperature to 30°C markedly increased the number of experiments yielding synaptic depression, suggesting that the underlying mechanism of iLTD may be heat sensitive.

iLTD is clearly different than the "passively propagated" LTD observed in s. oriens interneurons. The plasticity in this system occurs only at excitatory synapses onto pyramidal cells; these are then less effective at exciting interneurons via recurrent axon collaterals (Maccaferri and McBain, 1995). In our interneurons, the same stimulus that triggers potentiation in surrounding pyramidal cells clearly induces iLTD in s. radiatum interneurons. Recurrent collaterals from CA1 pyramidal cells are unlikely to contribute to the monosynaptic EPSC measured in the present work, since the EPSC peak precedes the peak of the pyramidal cell population EPSP. In fact, our data suggest that multiple excitatory synapses on a given interneuron are depressed after high frequency afferent stimulation, so that not only feedforward inhibition but also feedback inhibition can be affected by iLTD.

Mechanism of iLTD Is Distinct from That for Pyramidal Cell LTP or LTD

Two distinct mechanisms could account for iLTD. First, interneuron synapses may be depressed due to the activation of intracellular processes in the postsynaptic interneuron, like LTD in hippocampal pyramidal cells (Dudek and Bear, 1992; Mulkey and Malenka, 1992) or in cerebellar Purkinje cells (Ito et al., 1982; Linden et al., 1991). However, unlike LTD in these neurons, iLTD is not synapse specific, so that independent pathways onto the same interneuron are depressed following tetanus to just one pathway. A similar heterosynaptic depression has also been reported following tetanus to glutamatergic afferents in the neostriatum (Calabresi et al., 1992; Lovinger et al., 1993). The fact that iLTD is not synapse specific argues strongly that the locus of iLTD is not presynaptic, since it is difficult to imagine how one population of presynaptic fibers could communicate with an entirely separate, spatially distant population of presynaptic fibers. In addition, Ca^{2+} entry through NMDA channels is not sufficient to trigger iLTD, as interneuron LTD is not triggered after pairing membrane depolarization with afferent stimulation (a protocol that triggers robust LTP in pyramidal cells by maximizing Ca^{2+} entry through NMDA channels) (see also Maccaferri and McBain, 1996). In support of this, voltage clamping the neuron at negative potentials that minimize NMDA currents does not prevent iLTD.

A second possible explanation of our results is that tetanic stimulation produces an extracellularly released factor that triggers LTD. Recent evidence suggests that strong tetanic stimulation of s. radiatum can produce synaptic depression in unstimulated pathways onto pyramidal cells; like iLTD, this depression is heterosynaptic (Christie et al., 1994; Barr et al., 1995; Scanziani et al., 1996) and apparently requires activity in cells other than the recorded neuron (Scanziani et al., 1996). Scanziani et al. (1996) suggest that a soluble factor may be released extracellularly, causing nonspecific depression of glutamatergic transmission at local synapses. We currently favor the idea that this factor may also be responsible for the iLTD at interneuron synapses.

iLTD Contribution to the Development of Epileptiform Electrical Activity

We have identified a novel mechanism in hippocampus that weakens excitatory synapses onto GABAergic interneurons. Since interneurons control and synchronize the output of large groups of pyramidal cells, depression of multiple excitatory synapses onto an interneuron will disinhibit many pyramidal cells and disrupt function. Although we cannot say as yet under what physiological conditions iLTD is elicited, it is clearly observed after high frequency firing of afferents like that seen during epileptiform activity and is likely to contribute to or even underlie hippocampal disinhibition during epilepsy. One possibility might be that high frequency afferent activity allows a large Ca^{2+} influx through Ca^{2+}-permeable AMPA channels, present on at least some hippocampal interneurons (McBain and Dingledine, 1993).

iLTD may represent an early form of the dormant basket cell phenomenon, observed in animal models of epilepsy (Sloviter, 1991). Although dormant basket cells (interneurons) in epileptic tissue remain viable and can release GABA, they are functionally denervated or "dormant" (Sloviter, 1987; 1991). Disinhibition in dentate granule cells follows either kainic acid treatment (resulting in seizures) (Sloviter, 1992) or high frequency electrical stimulation (Stelzer et al., 1987; Scharfman and Schwartzkroin, 1990; Zhao and Leung, 1991), each

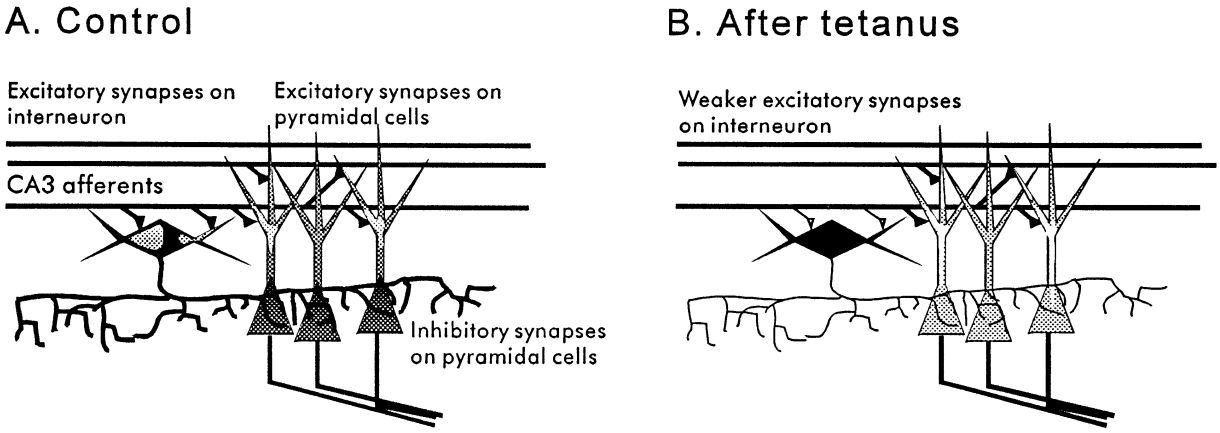

Figure 6. Diagram Illustrating the Consequences of iLTD on the Local Circuit in the CA1 Region

Interneurons of s. radiatum innervate CA1 pyramidal cells, releasing GABA either onto cell bodies or onto dendrites (not illustrated). Darker shading of the pyramidal cell represents hyperpolarization, while lighter shading represents depolarization.

(A) Excitatory afferents from CA3 release glutamate onto pyramidal cells and interneurons.

(B) After high frequency stimulation, LTP is induced at some pyramidal cell synapses, but synapses on interneurons undergo LTD, making interneurons far less excitable, resulting in less GABA released onto their pyramidal cell targets. Through these complimentary mechanisms, CA1 pyramidal cells become more excitable than before high frequency afferent activity.

of which produces patterns of hippocampal sclerosis resembling that seen in human temporal lobe epilepsy (Bruton, 1988). The original dormant basket cell hypothesis included the idea that presynaptic excitatory cells simply die in epilepsy, leaving a normal inhibitory cell without innervation (Franck et al., 1988; Sloviter, 1991; Nakajima et al., 1991). Other data suggest instead that interneurons become less excitable after high frequency stimulation or after kainate lesions of CA3 (Franck et al., 1988; Scharfman and Schwartzkroin, 1990; Nakajima et al., 1991). Our results show directly that interneuron responsiveness is decreased within seconds after high frequency stimulation without death of afferents. iLTD may thus produce functional denervation of hippocampal interneurons as a consequence of epileptiform bursting.

Conclusions

Because feedforward interneurons innervate hundreds of pyramidal cells, induction of iLTD in a single interneuron will increase the excitability of large groups of pyramidal cells in the local area (Figure 6). By reducing local inhibition, excitatory synapses on pyramidal cells that have been potentiated will be more effective at driving CA1 pyramidal cells to threshold, powerfully enhancing excitation in the local region.

Experimental Procedures

Recordings and Analysis

Sprague–Dawley rats (16–26 days old) were decapitated following deep halothane anesthesia. The brain was rapidly removed and placed in ice-cold ACSF (mM): NaCl, 119; $NaHCO_3$, 26; KCl, 2.5; NaH_2PO_4, 1; $CaCl_2$, 2.5; $MgSO_4$, 1.3; and glucose, 10; saturated with 95% O_2 and 5% CO_2 (pH 7.4). Coronal slices (400 μm) were cut immediately from the dorsal hippocampus using a vibratome. After cutting, slices were held for 1–5 hr in an interface chamber at room temperature and transferred to a recording chamber where the slice was held submerged between two nets. The recording solution was identical to ACSF except that $CaCl_2$ was increased to 4 mM, $MgSO_4$ was replaced by 4 mM $MgCl_2$, and 100 μM picrotoxin was included; the bath temperature was maintained at 29–31°C. The CA3 region of the slice was removed using a scalpel cut to prevent epileptiform bursting.

Tight-seal whole-cell recordings were obtained from the cell bodies of interneurons located in s. radiatum or from pyramidal cells in the CA1 region (McBain et al., 1994). Patch electrodes were pulled from borosilicate glass and had resistances of 2–5 MΩ when filled with (mM): potassium gluconate, 100; EGTA, 0.6; Na–GTP, 0.3; Na–ATP, 2; $MgCl_2$, 5; HEPES, 40; and biocytin, 0.4%. For voltage-clamp recordings, 100 mM cesium gluconate was substituted for potassium gluconate. The patch pipet was positioned under visual control within s. radiatum using a Zeiss water immersion 40 × objective with Hoffman-modified optics. Recordings were made using an Axopatch 200 or an Axoclamp 2A, and cells were either voltage clamped to −85 mV or held at −85 mV in bridge mode by passage of current into the electrode. Input resistance ranged from 200–700 Mohms, and series resistance ranged from 10–30 Mohms. Input resistance and series resistance were carefully monitored on-line, and experiments were discarded if changes >10% were seen. Field extracellular recordings were made by placing a microelectrode (~1 MΩ) filled with 2 M NaCl into s. radiatum close to the interneuron and approximately "on-beam" with the stimulating electrode.

Records were filtered at 1–2 kHz and digitized at 5–10 kHz on an 80486 personal computer using software written in the Axobasic programming environment and kindly donated by Drs. Daniel Madison and Felix Schweizer. The maximal initial slope of field EPSPs and intracellularly recorded EPSPs, and the peak amplitudes of voltage-clamped EPSCs were measured. No differences in iLTD were observed between current-clamp and voltage-clamp recordings, and data using each recording mode have been pooled in the figures, using the percent change in EPSP slope and the percent change in EPSC amplitude (since EPSC amplitude is directly proportional to slope). Baseline values were defined as the mean value of the EPSC amplitude or EPSP slope during the 2 min period, 3 min prior to tetanus.

Electrical Stimulation

A bipolar stimulating electrode was placed in s. radiatum to activate Shaffer collaterals of the CA3 pyramidal cells at 0.2 Hz. The evoked EPSC or EPSP often included late polysynaptic events that likely result from CA1 recurrent collaterals excited by CA3 afferents (Figures 1B and 4D); only the early monosynaptic event was measured (see Figure 1C). Monosynaptic events in the presence of picrotoxin

were entirely abolished in CNQX and d,l-APV, demonstrating that they result from activation of glutamatergic fibers (unpublished data). The latency to onset of EPSPs and EPSCs was measured by hand from the center of the stimulus artifact to the event onset. Reported values are the means ± the SEM. Latencies for EPSCs in voltage-clamp experiments ranged from 1.2–5.3 ms; corresponding field potential onsets ranged from 2.7–6.5 ms. Latencies for EPSPs in current-clamp experiments ranged from 1.4–4.3 ms; corresponding field potential onsets ranged from 2.3–5.6 ms.

After a stable baseline period of 7–20 min, afferents were stimulated twice at 100 Hz for 1 s with a 20 s interval between trains; the intensity was increased 1.5-fold during tetanic stimulation. We routinely depolarized the neuron during tetanic stimulation when the experiment was carried out in voltage clamp, to permit the membrane to depolarize during the tetanus. "Pairing" was achieved by depolarizing the cell to approximately −10 mV during 60 afferent stimuli at test intensity at 1 Hz. Of 13 pairing experiments, 4 were carried out using the perforated patch technique (0.25 μg/ml amphotericin added to potassium gluconate internal solution). The general health of the interneuron did not change after pairing or tetanus, as assessed by measurements of input resistance and action potential shape.

To stimulate two afferent pathways, a second stimulating electrode was placed on the side of the interneuron opposite that of the first stimulating electrode. The average distance between stimulating electrodes as measured post hoc in fixed slices was 850 μm. Independence was assessed by testing for paired-pulse facilitation between the two pathways (interpulse interval, 30 ms).

Biocytin Processing

Immediately after use, slices were fixed in 4% paraformaldehyde and resectioned at 75 μm. The sections were reacted as described with avidin–horseradish peroxidase (McBain et al., 1994). Sections were mounted on slides, and camera lucida drawings were made, collapsing the three-dimensional morphology into a line drawing in two dimensions.

Calbindin Immunocytochemistry

After a 1 hr rest period, freshly cut hippocampal slices were fixed overnight in 4% paraformaldehyde. Fixed slices were incubated in 0.2 M glycine followed by 30% sucrose and serially resectioned at 30 μm. Slices were then preincubated for 2 hr in 3% normal goat serum with triton-X and overnight in primary rabbit anti-calbindin antibody (1:500;) at 4°C. Following this, the slices were incubated (for 1 hr) in a secondary donkey anti-rabbit antibody (1:100) conjugated to rhodamine for visualization. Antibodies were obtained from Swant, Switzerland.

Acknowledgments

The authors thank Scott Douglas for histological and immunocytochemical staining of interneurons, and Drs. Lawrence Katz, Donald Lo, Felix Schweizer, and John H. Williams for helpful comments on the manuscript. This work was supported by NIH grants NS30500 to J. A. K. and NRSA NS09734 to L. L. M.

Received July 26, 1996; revised December 24, 1996.

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