Activity-Dependent Depression of Local Excitatory Connections in the CA1 Region of Mouse Hippocampus

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Fink AE, Sarinana J, Gray EE, O'Dell TJ. Activity-dependent depression of local excitatory connections in the CA1 region of mouse hippocampus. J Neurophysiol 97: 3926-3936, 2007. First published April 4, 2007; doi:10.1152/jn.00213.2007. The existence of recurrent excitatory synapses between pyramidal cells in the hippocampal CA1 region has been known for some time yet little is known about activity-dependent forms of plasticity at these synapses. Here we demonstrate that under certain experimental conditions, Schaffer collateral/commissural fiber stimulation can elicit robust polysynaptic excitatory postsynaptic potentials due to recurrent synaptic inputs onto CA1 pyramidal cells. In contrast to CA3 pyramidal cell inputs, recurrent synapses onto CA1 pyramidal cells exhibited robust pairedpulse depression and a sustained, but rapidly reversible, depression in response to low-frequency trains of Schaffer collateral fiber stimulation. Blocking GABA_B receptors abolished paired-pulse depression but had little effect on low-frequency stimulation (LFS)-induced depression. Instead, LFS-induced depression was significantly attenuated by an inhibitor of A1 type adenosine receptors. Blocking the postsynaptic effects of GABA_B and A1 receptor activation on CA1 pyramidal cell excitability with an inhibitor of G-protein-activated inwardly rectifying potassium channels had no effect on either pairedpulse depression or LFS-induced depression. Thus activation of presynaptic GABA_B and adenosine receptors appears to have an important role in activity-dependent depression at recurrent synapses. Together, our results indicate that CA3-CA1 and CA1-CA1 synapses exhibit strikingly different forms of short-term synaptic plasticity and suggest that activity-dependent changes in recurrent synaptic transmission can transform the CA1 region from a sparsely connected recurrent network into a predominantly feedforward circuit.

INTRODUCTION

Recurrent excitatory synaptic connections between pyramidal cells are a common feature of microcircuits within many regions of the neocortex and are thought to have an important role in the synchronization of neuronal activity in both normal physiological states (Silberberg et al. 2005; Traub et al. 2004) and in pathophysiological conditions such as epilepsy (Jin et al. 2006; Smith and Dudek 2002). Within the hippocampus, recurrent excitatory synapses are most prominent between CA3 pyramidal cells (Ishizuka et al. 1990; Miles and Wong 1986, 1987) and the high degree of local circuit connectivity provided by these synapses is thought to enable the hippocampal CA3 region to function as a center for autoassociative memory (see Rolls and Kesner 2006 for review). In contrast, relatively few recurrent excitatory synaptic connections are thought to exist between pyramidal cells in the hippocampal CA1 region (Deuchars and Thomson 1996; Knowles and Schwartzkroin 1981). Instead, the output of CA1 pyramidal cells primarily regulates the activity of local inhibitory interneurons and neurons in brain structures outside the hippocampus (Amaral 1993; Cenquizca and Swanson 2006; Freund and Buzsaki 1996).

Hippocampal CA1 pyramidal cells are not devoid of recurrent excitatory synaptic connections however. Although much less common than in the CA3 region, recurrent synapses between CA1 pyramidal cells have been demonstrated in a number of studies (Aniksztejn et al. 2001; Christian and Dudek 1988; Crépel et al. 1995; Deuchars and Thomson 1996; Klishin et al. 1995; Mlinar et al. 2001; Radpour and Thomson 1991; Shao and Dudek 2004). Importantly, the properties of these synapses appear to be very different from Schaffer collateral/ commissural fiber synapses arising from CA3 pyramidal cells. For example, although CA3 pyramidal cell synapses onto CA1 pyramidal cells exhibit robust paired-pulse facilitation, paired recordings of CA1-CA1 synapses have shown that recurrent synapses exhibit paired-pulse depression (Deuchars and Thomson 1996). This suggests that the basal probability of transmitter release is strikingly different at these two synapses. Moreover, recurrent excitatory synapses between CA1 pyramidal cells are more sensitive to the inhibitory effects of adenosine (Klishin et al. 1995) and serotonin (Mlinar et al. 2001, 2003), suggesting that Schaffer collateral and recurrent synaptic inputs onto CA1 pyramidal cells are uniquely regulated by different neuromodulators.

Like Schaffer collateral fiber synapses onto CA1 pyramidal cells, recurrent synaptic connections between CA1 pyramidal cells are mediated by both AMPA- and NMDA-type glutamate receptors (Crépel et al. 1997; Deuchars and Thomson 1996). The presence of NMDA receptors at recurrent synaptic connections suggests that these synapses may exhibit NMDA receptor-dependent forms of synaptic plasticity. Consistent with this, activity-dependent changes in the strength of recurrent synaptic connections between CA1 pyramidal cells are thought to have a crucial role in the generation of β -frequency oscillations of neuronal activity in the hippocampal CA1 region (Traub et al. 2004; Whittington et al. 1997). Moreover, recurrent synapses between CA1 pyramidal cells are enhanced in experimental models of epilepsy (Shao and Dudek 2004; Smith and Dudek 2001, 2002). Thus activity-dependent changes in synaptic strength at recurrent synapses in the CA1 region are likely to be involved in both normal hippocampal

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function and in pathophysiological states. It is not yet known, however, whether recurrent synapses in the CA1 region exhibit long-term potentiation and/or long-term depression. Moreover, the cellular mechanisms that might underlie either long or short-term forms of plasticity at these synapses are unclear. This is most likely due to the fact that the relatively sparse synaptic connections between CA1 pyramidal cells make experimental studies of these synapses exceedingly difficult.

Here we describe the results of studies examining plasticity at synaptic connections between CA1 pyramidal cells. We find that under certain experimental conditions Schaffer collateral/ commissural fiber activation can elicit surprisingly robust polysynaptic excitatory postsynaptic potentials (EPSPs) due to activation of recurrent connections between CA1 pyramidal cells. To determine whether recurrent synapses onto CA1 pyramidal cells exhibit short-term forms of synaptic plasticity, we examined how activating networks of interconnected pyramidal cells in the hippocampal CA1 region with different patterns of Schaffer collateral/commissural fiber stimulation modulates polysynaptic EPSPs. In contrast to CA3 pyramidal cell inputs, recurrent synapses onto CA1 pyramidal cells exhibit strong, GABA_B receptor-mediated paired-pulse depression. In addition, short trains of low-frequency stimulation (LFS) that have no effect on transmission at Schaffer collateral fiber synapses induced a profound, but rapidly reversible, depression of polysynaptic EPSPs that was mediated, in part, by activation of A1 type adenosine receptors. Our results thus indicate that CA1-CA1 recurrent synapses exhibit forms of short-term synaptic plasticity that are strikingly different from CA3 pyramidal cell synapses onto CA1 pyramidal cells.

METHODS

Slice preparation and extracellular recordings

Standard techniques were used to prepare 400 µm thick hippocampal slices from hippocampi obtained from halothane anesthetized, 8 -16 wk old, male C57Bl/6 mice (Charles River Laboratories, Wilmington, MA). All techniques were approved by the UCLA Institutional Animal Care and Use Committee. Briefly, transverse slices were cut approximately parallel to the alvear fibers using a manual tissue chopper and the CA3 region was completely removed. Slices were then maintained at 30°C in an interface-slice type recording chamber perfused ($\approx 2-3$ ml/min) with an oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) containing 124.0 mM NaCl, 4.4 mM KCl, 25 mM NaHCO₃, 1.0 mM NaH₂PO₄, 2.0 mM CaCl₂, 1.2 mM MgSO₄, and 10 mM glucose and allowed to recover for ≥ 1 h prior to an experiment. All experiments were done at 30°C. For recordings, a slice was transferred into a submerged-slice recording chamber perfused with ACSF containing 100 µM picrotoxin to block GABA_A receptor-mediated inhibitory synaptic transmission. A bipolar, nichrome wire stimulating electrode was placed in stratum radiatum of the hippocampal CA1 region to activate Schaffer collateral/ commissural fiber synapses (hereafter referred to simply as Schaffer collateral fibers) and an extracellular glass microelectrode filled with ACSF (resistance = 5–10 M Ω) was used to record field excitatory postsynaptic potentials (fEPSPs). At the start of each experiment the strength of presynaptic fiber stimulation was adjusted to evoke fEPSPs with durations of \geq 50 ms from onset (stimulation rate = 0.02 Hz).

Whole cell current clamp recordings and data analysis

Whole cell current clamp recordings were performed using low resistance $(2 - 6 \text{ M}\Omega)$ patch electrodes filled with a solution contain-

ing 120.0 mM potassium gluconate, 20.0 mM KCl, 2.0 mM MgCl₂, 4.0 mM Na₂-ATP, 0.3 mM Tris-GTP, 14 mM phosphocreatine, and 10 mM HEPES (pH = 7.3). If needed, constant injections of hyperpolarizing current were used to maintain membrane potentials between -65 and -75 mV and 50 ms long pulses of hyperpolarizing current (0.1 nA) were injected every 20 s to monitor both series and input resistance. In most experiments we attempted to adjust the intensity of Schaffer collateral fiber stimulation to a level that evoked polysynaptic EPSPs that were below threshold for postsynaptic action potential generation. In many cells, however, robust polysynaptic EPSPs that evoked action potentials were elicited by even the lowest stimulation strengths that evoked polysynaptic responses. In all figures shown below action potentials are truncated for clarity. During baseline recordings presynaptic fiber stimulation pulses were delivered once every 20 s.

Data acquisition and analysis were performed using either the Experimenter's Workbench/Common Processing software package from DataWave Technologies (Longmont, CO) or PClamp (Molecular Devices, Sunnyvale, CA). In both field and whole cell recordings we used the area under the postsynaptic response (response onset to return to resting membrane potential) to measure the magnitude of polysynaptic EPSPs. In some experiments the initial rising slope of EPSPs was used to measure the magnitude of monosynaptic inputs. Paired and unpaired *t*-test or, where appropriate, one-way repeated measure ANOVAs followed by Bonferroni *t*-test for repeated comparisons to control were used to determine statistical significance.

8-cyclopentyl-1,3-dipropylxanthine (DPCPX), CGP55845, LY341495, SCH23390, isomoltane hemifumarate, CP93129 and D-APV were obtained from Tocris Bioscience (Ellisville, MO). All other compounds were obtained from Sigma-Aldrich (St. Louis, MO).

RESULTS

Extracellular recordings of polysynaptic responses in CA1

We first examined whether recurrent excitatory connections between hippocampal CA1 pyramidal cells could be reliably detected using extracellular recordings in slices where fast inhibitory synaptic transmission was blocked. Consistent with previous reports (Crépel et al. 1997), high-intensity Schaffer collateral fiber stimulation elicited prolonged fEPSPs in slices bathed in normal ACSF containing 100 µM picrotoxin and these responses were strongly depressed when polysynaptic transmission was suppressed by bath application of a high-Ca²⁺/Mg²⁺ ACSF containing 4.0 mM CaCl₂ and 6.0 mM MgSO₄ (Fig. 1A). To explore the potential contribution of polysynaptic EPSPs in these recordings in more detail we next examined whether two previously identified unique properties of CA1 recurrent synapses can be detected in extracellularly recorded responses elicited by Schaffer collateral fiber stimulation in dis-inhibited slices. First, paired recordings of CA1 to CA1 excitatory synapses have shown that recurrent synapses onto CA1 pyramidal cells exhibit pronounced paired-pulse depression (Deuchars and Thomnson 1996). Thus we measured fEPSPs elicited by pairs of presynaptic fiber stimulation pulses delivered with inter-pulse intervals of 200 - 800 ms. As shown in Fig. 1B, the prolonged fEPSPs elicited in the absence of inhibitory synaptic transmission exhibited paired-pulse depression at all intervals tested. Second, synaptic transmission at recurrent synapses onto CA1 pyramidal cells is facilitated during low-frequency trains of presynaptic action potentials (Radpour and Thomson 1991). Thus as another test of the potential contribution of polysynaptic EPSPs to responses elicited by Schaffer collateral fiber stimulation in dis-inhibited



FIG. 1. Extracellular recordings of postsynaptic responses elicited by Schaffer collateral fiber stimulation in dis-inhibited slices. A: blocking polysynaptic transmission with elevated concentrations of external divalent cations (indicated by the bar) inhibits field excitatory postsynaptic potential (fEPSP) area. fEPSP area was reduced to $59 \pm 2\%$ of baseline 10 min after application of artificial cerebrospinal fluid (ACSF) containing 4.0 mM $CaCl_2$ and 6.0 mM MgSO₄ (n = 4, P < 0.001 compared with baseline). Picrotoxin (100 μ M) was present throughout the experiment. Inset: fEPSPs recorded during baseline and 10 min after application of ACSF containing elevated concentrations of Ca²⁺ and Mg²⁺. Calibration bars are 10 ms and 1 mV. B: fEPSPs elicited by Schaffer collateral fiber stimulation in the presence of picrotoxin exhibit paired-pulse depression. Each point shows the mean \pm SE paired-pulse ratio (area of the 2nd response/area of the 1st response) for pairs of presynaptic fiber stimulation pulses delivered with inter-pulse intervals of 200, 400, 600, and 800 ms (n = 5). C: fEPSP area is suppressed during low-frequency trains of Schaffer collateral fiber stimulation in slices bathed in ACSF containing 100 μ M picrotoxin (n = 13). Inset: fEPSPs recorded during baseline and at the end of a 30 s long train of 1 Hz stimulation. Calibration bars are 10 ms and 1 mV.

slices we examined whether the area of fEPSPs were enhanced during short trains of LFS. Surprisingly, we found that synaptic responses underwent a rapid-onset but modest depression at the start of a 30 s long train of 1 Hz that persisted for approximately ~ 10 s (fEPSP area was reduced to 83 \pm 2% of baseline, n = 13, P < 0.001 compared with baseline). A slower onset, but more profound suppression then developed throughout the rest of the train (after 30 s of 1 Hz stimulation fEPSP area was reduced to $51 \pm 3\%$ of baseline, P < 0.001 compared with baseline). LFS did not, however, induce a lasting depression of fEPSP area and responses rapidly returned to baseline levels (5 min post1Hz stimulation fEPSP area was $94 \pm 3\%$ of baseline). Thus while the inhibition of fEPSP area induced by high-Ca²⁺/Mg²⁺ACSF and the presence of paired-pulse depression are consistent with the idea that polysynaptic EPSPs contribute to postsynaptic responses generated by Schaffer collateral fiber stimulation when inhibitory synaptic transmission is blocked, the LFS-induced suppression of postsynaptic responses appears to be inconsistent with previously identified properties of recurrent excitatory synaptic connections between CA1 pyramidal cells.

Schaffer collateral fiber stimulation elicits polysynaptic EPSPs due to CA1-CA1 synapses when $GABA_A$ receptormediated inhibitory synaptic transmission is blocked

Although extracellular recordings have been used by others to study polysynaptic EPSPs in the hippocampal CA1 region (Crépel et al. 1997), the postsynaptic responses recorded with extracellular electrodes under our experimental conditions are complex and not only reflect EPSPs but also postsynaptic cell firing. These responses are thus difficult to interpret and the potential contribution of polysynaptic EPSPs to the evoked responses is unclear. Because extracellular recordings seemed less then ideal for studying recurrent synaptic connections between CA1 pyramidal cells we turned to whole cell currentclamp recordings from individual CA1 pyramidal cells to examine whether Schaffer collateral fiber stimulation can elicit polysynaptic responses in CA1 pyramidal cells. As shown in Fig. 2A, intensities of presynaptic fiber stimulation that elicited monosynaptic EPSPs under control conditions elicited numerous polysynaptic EPSPs following bath application of picrotoxin. In addition, the latency to peak depolarization of the polysynaptic responses was decreased by increases in presynaptic fiber stimulation intensity (Fig. 2B), a hallmark of polysynaptic EPSPs recorded in the hippocampal CA1 region (Crépel et al. 1997; Mlinar et al. 2001). In the presence of picrotoxin Schaffer collateral fiber stimulation-evoked polysynaptic EPSPs like those shown in Fig. 2, A and B were surprisingly common and observed in virtually every cell tested (126 of 135 cells).

Polysynaptic EPSPs evoked by Schaffer collateral fiber stimulation in CA1 mini-slices lacking the dentate gyrus and subiculum as well as the CA3 region were indistinguishable from those seen in control slices where just the CA3 region was removed (Fig. 2*B*). This indicates that the polysynaptic EPSPs recorded in our experiments are not due to synaptic inputs onto CA1 pyramidal cells arising from the subiculum (Berger et al. 1980; Commins et al. 2002; Harris et al. 2001; Knopp et al. 2005) but are instead due to local excitatory connections between CA1 pyramidal cells. Moreover, activation of 5-HT_{1B}



FIG. 2. Blocking GABA_A-mediated inhibitory synaptic transmission reveals polysynaptic EPSPs due to recurrent synaptic connections between CA1 pyramidal cells. A: examples of postsynaptic responses elicited by Schaffer collateral fiber stimulation recorded in the same cell before (baseline) and after bath application of 100 μ M picrotoxin. Calibration bars are 10 ms and 5 mV. Similar responses were seen in 5 separate experiments. B: examples of postsynaptic responses elicited by Schaffer collateral fiber stimulation at 4 different stimulation intensities in control cells (CA3 region removed) and in cells in isolated CA1 mini-slices. Calibration bars are 20 ms and 10 mV. Picrotoxin (100 μ M) was present throughout. Responses are representative of 6 control cells and 8 cells from CA1 mini-slices. C: bath application of the 5-HT_{1B} receptor agonist CP93129 (10 μ M, indicated by the bar) inhibits polysynaptic EPSPs. CP93129 reduced the area of postsynaptic potentials to $18 \pm 6\%$ of baseline, P < 0.001 compared with baseline, (n = 5). Inset: superimposed EPSPs recorded during baseline and 10 min post CP93129 application. Calibration bars are 15 ms and 5 mV. D: bath application of ACSF containing 4.0 mM CaCl2 and 6.0 mM MgSO4 (indicated by the bar) blocks polysynaptic EPSPs evoked in the presence of picrotoxin (n = 5). Inset: superimposed EPSPs evoked before (baseline) and after bath application of high Ca^{2+}/Mg^{2+} ACSF in a representative cell. Calibration bars are 10 ms and 5 mV. E: EPSPs elicited by 5 different intensities of Schaffer collateral fiber stimulation in a cell bathed in ACSF containing 2.4 mM KCl (left) and after bath application of ACSF containing 4.4 mM KCl (right). Note that polysynaptic EPSPs are much more prevalent in the presence of elevated concentrations of external K⁺. Calibration bars are 10 ms and 10 mV. Similar responses were recorded in 4 separate experiments.

receptors with CP93129 (10 μ M), which selectively inhibits local excitatory synapses between CA1 pyramidal cells (Mlinar et al. 2001, 2003), strongly suppressed polysynaptic EPSPs elicited by Schaffer collateral fiber stimulation (Fig. 2*C*). Finally, bath application of high-Ca²⁺/Mg²⁺ ACSF completely suppressed polysynaptic responses leaving only monosynaptic EPSPs (Fig. 2*D*). Thus in agreement with previous findings (Crépel et al. 1997; Mlinar et al. 2001, 2003), surprisingly robust network-driven polysynaptic EPSPs due to CA1CA1 recurrent synaptic connections can be elicited in hippocampal CA1 pyramidal cells when GABA_A receptor-mediated inhibitory synaptic transmission is blocked.

Paired recordings searching for CA1-CA1 pyramidal cell synaptic connections have revealed very low connection rates ranging from 0 to 1% (Deuchars and Thomson 1996Knowles and Schwartkroin 1981) and it was thus surprising that such robust polysynaptic EPSPs could be so readily elicited by Schaffer collateral fiber stimulation in our experiments. One feature of our experimental conditions that could facilitate CA1 pyramidal cell firing in response to Schaffer collateral fiber activation, and thus enhance detection of polysynaptic EPSPs due to recurrent synapses, is the somewhat high concentrations of KCl (4.4 mM) used in our ACSF. We thus performed a limited number of experiments comparing postsynaptic responses elicited by Schaffer collateral fiber stimulation in cells first bathed in a modified ACSF containing 2.4 mM KCl and then bathed in ACSF containing 4.4 mM KCl (100 μ M picrotoxin present in both conditions). As shown in Fig. 2E, clear polysynaptic EPSPs were rarely observed in cells bathed in ACSF containing 2.4 mM KCl while robust polysynaptic EPSPs could be detected following application of ACSF containing 4.4 mM KCl. This suggests that slightly elevated concentrations of extracellular K⁺ facilitates detection of EPSPs due to recurrent CA1-CA1 pyramidal cell synapses; most likely because it increases CA1 pyramidal cell excitability and thus enhances CA1 pyramidal cell firing in response to activation of Schaffer collateral fiber inputs.

Short-term plasticity at recurrent synapses onto CA1 pyramidal cells

To determine whether recurrent excitatory synapses in the hippocampal CA1 region exhibit short-term forms of synaptic plasticity we examined the effects of pairs of presynaptic fiber stimulation pulses delivered with inter-pulse intervals of 200 - 800 ms. Consistent with the results from our extracellular recording experiments, polysynaptic EPSPs exhibited robust paired-pulse depression at all inter-pulse intervals tested (Fig. 3*A*). Indeed, at the shortest inter-pulse interval tested polysynaptic EPSPs appeared to be completely suppressed and only monosynaptic EPSPs were evoked by the second stimulation pulse (Fig. 3*A*). In contrast, monosynaptic Schaffer collateral fiber inputs onto CA1 pyramidal cells recorded in slices where polysynaptic EPSPs were blocked by bath application of high-Ca²⁺/Mg²⁺ ACSF exhibited no paired-pulse depression over these same inter-pulse intervals (Fig. 3*B*).

We also examined short-term plasticity of polysynaptic EPSPs by using a short train of LFS. As we observed in our extracellular recordings, a 30 s long train of 1 Hz Schaffer collateral fiber stimulation had two distinct effects on polysynaptic EPSPs (Fig. 4). At the beginning of the 1-Hz train polysynaptic EPSPs underwent a modest depression that persisted for 5–10 s (EPSP area was reduced to 75 ± 8% of baseline, n = 5, P < 0.05 compared with baseline). A more profound suppression then slowly developed throughout the rest of the train such that by the end of the 1 Hz stimulation train only monosynaptic EPSPs remained (EPSP area was reduced to $6 \pm 1\%$ of baseline, P < 0.001 compared with baseline). This depression was short-lasting, however, and polysynaptic EPSPs rapidly reemerged after the 1 Hz stimula-



FIG. 3. Polysynaptic EPSPs recorded in the presence of picrotoxin exhibit paired-pulse depression. A: pairs of presynaptic fiber stimulation pulses were delivered with interpulse intervals of 200, 400, 600, and 800 ms. Note the pronounced paired-pulse depression (ratio = area of 2nd response \div area of 1st response \times 100) (n = 5). Inset: example of responses elicited by pairs of stimulation pulses (200-800 ms) in a representative cell. Calibration bars are 5 mV and 50 ms. The expanded region shows a monosynaptic EPSP elicited by the 2nd presynaptic fiber stimulation pulse delivered 200 ms after the 1st (calibration bars are 1 mV and 10 ms). B: neither the area (\bullet) nor the slope (\odot) of monosynaptic EPSPs elicited by Schaffer collateral fiber stimulation exhibit paired-pulse depression (n = 6). Slices were bathed in high Ca²⁺/Mg²⁺ ACSF to block polysynaptic EPSPs. Inset: EPSPs elicited by pairs of presynaptic fiber stimulation pulses delivered with interpulse intervals of 200-800 ms in a representative cell. Calibration bars are 5 mV and 50 ms.

tion train (two minutes post1 Hz stimulation EPSP area was $101 \pm 4\%$ of baseline, Fig. 4*B*). In contrast, the monosynaptic component of the postsynaptic responses measured by the initial slope of the EPSPs was facilitated at the start of the 1 Hz stimulation and never depressed below pre1 Hz baseline levels (Fig. 4*C*). This indicates that monosynaptic Schaffer collateral fiber synapses onto CA1 pyramidal cells are not depressed during 1 Hz stimulation. Consistent with this notion, 1 Hz stimulation also had no effect on monosynaptic Schaffer collateral fiber EPSPs recorded in slices where bath application of high-Ca²⁺/Mg²⁺ ACSF was used to block polysynaptic EPSPs (Fig. 4*D*).

Because the ionic composition of ACSF has been shown to strongly regulate the activity of recurrent excitatory synapses in visual cortex (Sanchez-Vives and McCormick 2000) we next examined whether both paired-pulse and LFS-induced depression of polysynaptic EPSPs could be observed under ionic conditions that more closely match those found in vivo. As shown in Fig. 4, *E* and *F*, polysynaptic EPSPs were strongly



FIG. 4. Low-frequency Schaffer collateral fiber stimulation inhibits recurrent synapses onto CA1 pyramidal cells. A: EPSPs recorded from a CA1 pyramidal cell before (baseline) and at the indicated time points during 1-Hz stimulation. Calibration bars are 5 mV and 25 ms. Note that only a monosynaptic EPSP remains by the end of the 1-Hz train. B: summary of 5 experiments like that shown in A. C: statistical analysis of 1-Hz stimulation-induced changes in synaptic responses. Polysynaptic EPSPs (area) are significantly suppressed at all 3 time points examined (*P < 0.05 compared with baseline, #P < 0.001 compared with baseline), whereas the monosynaptic component of the postsynaptic response (measured by the initial slope of the EPSP) is enhanced after 10 s of 1-Hz stimulation and not different from baseline at later time points. D: 1-Hz stimulation has no effect on either the area (•) or slope (\odot) of monosynaptic EPSPs recorded in cells where high Ca²⁺/Mg²⁻ ACSF was used to block polysynaptic EPSPs (n = 8). Inset: superimposed EPSPs recorded during baseline and at the end of the 1-Hz train. Calibration bars are 5 mV and 10 ms. E: polysynaptic EPSPs recorded in cells bathed in physiological ACSF (1.2 mM Ca²⁺, 1.0 mM Mg²⁺, and 3.5 mM K⁺) are strongly depressed by a 30-s-long train of 1-Hz stimulation (n = 9). F: statistical analysis of 1-Hz stimulation-induced changes in synaptic responses recorded in cells bathed in physiological ACSF. Polysynaptic EPSPs (D) are significantly suppressed at all 3 time points (*P < 0.05 and #P < 0.001 compared with baseline) while the monosynaptic component of the postsynaptic responses (initial slope, ■) is not affected. Inset: superimposed EPSPs recorded during baseline and at the end of the 1-Hz train. Calibration bars are 10 mV and 15 ms.

depressed during a 30 s long train of 1 Hz stimulation in slices bathed in a "physiological" ACSF containing 1.2 mM Ca²⁺, 1.0 Mg²⁺, and 3.5 mM K⁺ (Sanchez-Vives and McCormick 2000). In contrast, the initial slope of the postsynaptic EPSPs was unchanged during 1 Hz stimulation (Fig. 4F). Thus as we observed in cells bathed in our standard ACSF, a short train of 1 Hz stimulation has no effect on the monosynaptic, Schaffer collateral fiber inputs onto CA1 pyramidal cells bathed in physiological ACSF. We also observed robust paired-pulse depression of polysynaptic EPSPs in slices bathed in physiological ACSF. In these experiments the area of postsynaptic responses elicited by the second of two Schaffer collateral fiber stimulation pulses delivered with an inter-pulse interval of 500 ms was reduced $20 \pm 4\%$ of the first response in cells bathed in physiological ACSF (n = 9) and was reduced to $22 \pm 5\%$ of the first response in cells bathed in our standard ACSF (n =8). Together, these findings indicate that recurrent synapses between CA1 pyramidal cells exhibit robust activity-dependent depression under ionic conditions that mimic those found in vivo.

Paired-pulse depression of recurrent synapses is mediated by $GABA_B$ receptor activation

Activation of inhibitory interneurons by Schaffer collateral fiber stimulation can elicit GABA_B receptor-mediated slow inhibitory postsynaptic potentials (IPSPs) in CA1 pyramidal cells that can last for several hundred milliseconds and strongly inhibit pyramidal cell firing (Dutar and Nicoll 1988). Because the polysynaptic EPSPs evoked by Schaffer collateral fiber stimulation arise from a network of excitatory connections between CA1 pyramidal cells, it seemed likely that decreases in CA1 pyramidal cell firing due to slow IPSPs could importantly contribute to activity-dependent changes in recurrent synaptic connections between CA1 pyramidal cells. Indeed, in most cells a small, but clear, hyperpolarization lasting hundreds of milliseconds was present following polysynaptic EPSPs evoked by Schaffer collateral fiber stimulation (Fig. 5A). Consistent with the identification of these hyperpolarizations as slow IPSPs, they were completely abolished by the GABA_B receptor antagonist CGP55845 (Fig. 5A). Thus to determine whether slow IPSPs contribute to paired-pulse depression of polysynaptic EPSPs we next examined the effects of bath applied CGP55845 (5 μ M) on the paired-pulse depression of polysynaptic EPSPs induced by pairs of Schaffer collateral fiber stimulation pulses (inter-pulse interval = 500ms) delivered once every 20 s. As shown in Fig. 5, B and C, CGP55845 (5 μ M) completely abolished paired-pulse depression of polysynaptic EPSPs. Paired-pulse ratios (area of the 2nd response/area of the 1st response x 100) were $25 \pm 5\%$ during baseline and were $123 \pm 11\%$ 5 min post CGP55845 application (n = 8, P < 0.001). Paired-pulse depression of polysynaptic EPSPs induced by pairs of stimulating pulses delivered with inter-pulse intervals of 200 to 800 ms was also blocked in slices continuously bathed in ACSF containing 5 μ M CGP55845 (Fig. 6, A and B).

Importantly, activation of presynaptic $GABA_B$ receptors strongly suppresses glutamate release from presynaptic terminals in the hippocampal CA1 region (Isaacson et al. 1993; Lanthorn and Cotman 1981; Olpe et al. 1982), suggesting that



Sweep Number

FIG. 5. GABA_B receptor activation underlies paired-pulse depression of polysynaptic EPSPs. A: GABA_B receptor-mediated slow inhibitory postsynaptic potentials (IPSPs) are elicited by presynaptic fiber stimulation strengths that elicit polysynaptic EPSPs. The traces show superimposed postsynaptic responses (average of 3 responses) elicited by Schaffer collateral fiber stimulation before and after bath application of the GABA_B receptor antagonist CGP55845 (5 μ M). Calibration bars are 5 mV and 50 ms. *Right*: peak amplitude of the slow IPSPs before (open bars) and after (filled bars) bath application of CGP55845 (n = 7, asterisk, P < 0.001 compared with control). *B*: examples of EPSPs evoked by pairs of presynaptic fiber stimulation pulses (interpulse interval = 500 ms) in the same cell before (gray line) and after a 5-mi bath application of 5 μ M CGP55845 (dark line). Calibration bars are 5 mV and 50 ms. *C*: summary of 8 experiments like that shown in *B* where paired-pulse depression of polysynaptic EPSPs was examined before and during bath application of 5 μ M CGP55845 (indicated by the bar).

presynaptic GABA_B receptor activation might also contribute to paired-pulse depression of polysynaptic EPSPs. Indeed, the time course of paired-pulse depression of polysynaptic EPSPs was considerably longer than the time course of slow IPSPs (Fig. 7*A*), suggesting that slow IPSPs alone cannot account for paired-pulse depression of polysynaptic EPSPs. We thus next examined whether selectively blocking only the postsynaptic effects of GABA_B receptor activation with the G-protein-

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Inter-Pulse Interval (msec)

FIG. 6. Blocking GABA_B receptors with CGP55845 blocks paired-pulse depression of polysynaptic EPSPs. *A*: paired-pulse depression of polysynaptic EPSPs across a range of interpulse intervals (200–800 ms) in a control cell (ACSF +0.1% DMSO) and in a cell exposed to 5 μ M CGP55845. Calibration bars are 10 mV and 50 ms. *B*: summary of paired-pulse depression of polysynaptic EPSPs in 7 control cells (\circ , 0.1% DMSO) and 9 cells bathed in ACSF containing 5 μ M CGP55845 (\bullet , #*P* < 0.001, **P* < 0.01 compared with control).

activated, inwardly rectifying potassium (GIRK) channel blocker SCH23390 (Kuzhikandathil and Oxford 2002) mimicked the effects of CGP55845 on paired-pulse depression of polysynaptic EPSPs. Consistent with previous reports (Huang et al. 2005), SCH23390 (20 µM) strongly inhibited slow IPSPs recorded in cells where fast IPSPs were blocked with picrotoxin (100 μ M) and a combination of CNQX (25 μ M) and D-APV (50 μ M) was used to suppress excitatory synaptic transmission (Fig. 7B). SCH23390 had no effect, however, on paired-pulse depression of polysynaptic EPSPs (Fig. 7C). Thus while blocking all GABA_B receptors abolishes paired-pulse of depression of polysynaptic EPSPs, selectively blocking the effects of postsynaptic GABA_B receptor activation has no effect. This indicates that much of the paired-pulse depression of polysynaptic EPSPs is due to activation of presynaptic GABA_B receptors on recurrent synapses.

Adenosine receptor activation contributes to suppression of recurrent synapses during low-frequency Schaffer collateral fiber stimulation

To investigate whether $GABA_B$ receptor activation also contributes to the suppression of recurrent synapses during LFS we next examined the effects of blocking $GABA_B$ receptors with CGP55845 on the depression of EPSP area induced by a 30 s long train of 1 Hz stimulation. As shown in Fig. 8, *A* and *B*, the initial depression of EPSP area that occurs during



FIG. 7. The GIRK inhibitor SCH23390 strongly attenuates slow IPSPs (sIPSPs) but has no effect on paired-pulse depression of polysynaptic EPSPs. A: time course of paired-pulse depression of polysynaptic EPSPs does not match the time course of slow IPSPs. \bullet , mean \pm SE paired-pulse ratios for polysynaptic EPSPs (data from Figs. 3 and 5) plotted against the average slow IPSP (—) recorded in cells bathed in ACSF containing 100 μ M picrotoxin, 50 μ M D-APV and 25 μ M CNQX (n = 5, resting membrane potential = -64 mV, peak IPSP amplitude approximately equal to -6 mV). The residual EPSP is truncated. B: histograms show the mean \pm SE amplitude of slow IPSPs recorded during baseline (\Box) and 20 min after bath application of 20 μ M SCH23390 (■) in 5 separate experiments. SCH23390 significantly depressed both the peak IPSP amplitude (*left*, #P < 0.001 compared with baseline) and the amplitude of the IPSP measured 500 ms post stimulation (*right*, *P < 0.05compared with baseline). Inset: sIPSPs recorded in a cell bathed in ACSF containing picrotoxin (100 µM), CNQX (25 µM), and D-APV (50 µM) during baseline and 20 min post SCH23390 application. Calibration bars are 2 mV and 75 ms. C: 20-min bath application of SCH23390 (20 μ M, \blacksquare , n = 7) has no effect on paired-pulse depression of polysynaptic EPSPs (interpulse interval = 500 ms). \Box , paired-pulse depression in control cells (n = 8). Inset: paired pulse depression of polysynaptic EPSPs in a cell bathed in SCH23390. Calibration bars are 5 mV and 75 ms.



FIG. 8. Activation of GABA_B and A1 adenosine receptors mediates distinct phases of the suppression of recurrent synapses induced by 1-Hz stimulation. A: 30-s train of 1-Hz stimulation induces a robust suppression of polysynaptic EPSPs in both control cells (\bigcirc , 0.1% DMSO, n = 6) and in cells exposed to 5 μ M CGP55845 (\bullet , n = 10). B: statistical analysis of results shown in A. Although CGP55845 significantly attenuates the suppression of polysynaptic EPSPs seen at the start of 1-Hz stimulation (5 and 10 s), it had no effect on the amount of suppression seen at the end of 1-Hz stimulation (*P < 0.05, compared with control cells). The traces show EPSPs recorded before (baseline) and at the end of a 1-Hz train in a cell bathed in ACSF containing 5 μM CGP55845. Calibration bars are 5 mV and 25 ms. C: suppression of polysynaptic EPSPs during 1-Hz stimulation is attenuated in cells bathed in ACSF containing 200 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, \bullet , n = 8). \bigcirc , results from control cells (0.1% DMSO, n = 5). D: statistical analysis of results shown in C. Although blocking A1 receptors with DPCPX had no effect on the suppression of polysynaptic EPSPs seen at the start of 1-Hz stimulation (5 and 10 s of 1-Hz stimulation), it significantly inhibited the amount of suppression seen at later time points (#P < 0.001 compared with control). Inset: superimposed EPSPs recorded during baseline and after 30 s of 1-Hz stimulation in a control cell (left) and in a cell exposed to DPCPX (right). Calibration bars are 10 mV and 25 ms. E: G-protein-activated, inwardly rectifying potassium (GIRK) inhibitor SCH23390 (20 μ M) has no effect on the suppression of polysynaptic EPSPs induced by 1-Hz stimulation (\bullet , n = 9). \bigcirc , results from interleaved control experiments (n = 5). F: summary of 1-Hz stimulationinduced suppression of polysynaptic EPSPs at the indicated time points during the 1-Hz train in the presence and absence of SCH23390.

the first 10 s of 1 Hz stimulation was significantly attenuated in cells bathed in ACSF containing 5 μ M CGP55845. However, the more profound suppression of EPSP area that occurs later in the train was completely unaffected by CGP55845 (at the end of 1 Hz stimulation EPSP area was reduced to $10 \pm 2\%$ of baseline in control cells, n = 6, compared with $8 \pm 3\%$ of baseline in cells bathed in ACSF containing 5 μ M CGP55845, n = 10). Thus while GABA_B receptor activation appears to be responsible for the initial component of the depression of recurrent synapses onto CA1 pyramidal cells induced by 1 Hz stimulation, it has no role in the delayed component of the suppression that occurs later during the 1 Hz train.

Because activation of metabotropic glutamate receptors (mGluRs) can strongly suppress excitatory synaptic transmission in the hippocampus (Anwyl 1999) we next examined whether mGluR activation might contribute to LFS-induced depression of polysynaptic EPSPs. Bath application of the broad spectrum mGluR antagonist LY341495 (20 μ M) had no effect on LFS-induced depression (n = 8, data not shown), suggesting that mGluRs are not involved. Blocking mGluRs with LY341495 also had no effect on paired-pulse depression of polysynaptic EPSPs (n = 6, data not shown). Based on previous studies showing that activation of 5-HT_{1B} (Mlinar et al. 2001, 2003) and A1 type adenosine receptors (Klishin et al. 1995) can potently suppress recurrent synapses onto CA1 pyramidal cells we also examined whether 5-HT_{1B} or adenosine receptor activation might contribute to activity-dependent changes in synaptic transmission at these synapses. Bath application of the 5-HT_{1B} receptor antagonist isomaltane (10 μ M) had no effect on the depression of polysynaptic EPSPs during 1 Hz stimulation (n = 7, data not shown), suggesting that serotonin release does not contribute to the suppression of recurrent synapses during low-frequency stimulation. Blocking adenosine receptors with the A1 receptor selective antagonist DPCPX (200 nM) had no effect on paired-pulse depression of polysynaptic EPSPs (n = 7, data not shown) and also had no effect on the early component of the synaptic depression induced by 1 Hz stimulation (Fig. 8, C and D). The late component of the suppression induced by 1 Hz stimulation was, however, strongly attenuated by DPCPX. As shown in Fig. 8, C and D, EPSP area was reduced to $6 \pm 2\%$ of baseline at the end of 1 Hz stimulation in control cells (n = 5) while EPSP area was reduced to only $44 \pm 7\%$ of baseline in the presence of DPCPX (n = 8, P < 0.001 compared with control). This indicates that a significant component of the late phase of LFS-induced depression of recurrent synapses onto CA1 pyramidal cells is due to accumulation of extracellular adenosine and activation of A1 type adenosine receptors. Inhibiting GIRK channels with SCH23390 (20 µM) had no effect on 1 Hz stimulation-induced suppression of polysynaptic EPSPs (n = 9) (Fig. 8, E and F). This suggests that the effects of GABA and adenosine are not due to decreases in network excitability caused by activation of postsynaptic, GIRK channel-coupled receptors but are instead mediated by presynaptic receptors. Thus while activation of presynaptic GABA_B and A1 receptors contributes to the depression of recurrent synapses during LFS, our results indicate that GABA and adenosine mediate temporally distinct phases of the suppression of recurrent synapses during 1-Hz stimulation.

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DISCUSSION

Despite the fact that CA1 pyramidal cell synapses onto other CA1 pyramidal cells are rare (Deuchars and Thomson 1996; Knowles and Schwartkroin 1981), rough estimates suggest that a given CA1 pyramidal cell could receive potentially dozens of synaptic inputs from other CA1 pyramidal cells in a transverse hippocampal slice (Traub et al. 2004). Consistent with this, we find that polysynaptic EPSPs due to recurrent synaptic connections can be readily evoked in CA1 pyramidal cells by Schaffer collateral fiber stimulation in slices where fast GABA_A receptor-mediated inhibitory synaptic transmission is blocked, especially when neuronal excitability is enhanced by modestly elevated levels of extracellular K^+ .

Activity-dependent modulation of recurrent synaptic connections between CA1 pyramidal cells

Using these experimental conditions, we find that polysynaptic EPSPs elicited by Schaffer collateral fiber stimulation exhibit robust paired-pulse depression. The fact that presynaptic fiber stimulation strengths that elicited polysynaptic EPSPs also evoked slow IPSPs initially suggested to us that pairedpulse depression could result from slow IPSPs elicited by the first stimulation pulse that suppress CA1 pyramidal cell firing in response to subsequent pulses of presynaptic fiber stimulation. Consistent with this, blocking slow IPSPs with the GABA_B receptor blocker CGP55845 completely abolished paired-pulse depression of polysynaptic EPSPs. However, inhibiting sIPSPs with the GIRK channel blocker SCH23390 had no effect on paired-pulse depression. Thus rather than reflecting a network level phenomenon arising from slow IPSPinduced changes in CA1 pyramidal cell excitability, our results suggest that the paired-pulse depression is an intrinsic property of synaptic transmission at recurrent synapses mediated by presynaptic GABA_B receptors. Our results do not, however, rule out the possibility that postsynaptic GABA_B receptormediated slow IPSPs might also be involved, especially at shorter inter-pulse intervals where the slow IPSP is most robust.

Polysynaptic EPSPs due to recurrent synapses also underwent a profound short-term depression in response to short trains of 1-Hz Schaffer collateral fiber stimulation that exhibited at least two temporally and pharmacologically distinct phases. At the start of the LFS train there was a nearly immediate, but modest, suppression of polysynaptic EPSPs that persisted from \sim 5–10 s. This initial depression was then followed by a slower onset but more profound suppression of polysynaptic EPSPs such that by the end of the 1-Hz train, only monosynaptic EPSPs remained. Blocking GABA_B receptors completely abolished the initial phase of the LFS-induced depression suggesting that the cellular mechanisms responsible for this component of the depression are most likely the same as those that responsible for paired-pulse depression, i.e., decreases in glutamate release due to activation of presynaptic GABA_B receptors. The slower, but more robust, phase of synaptic depression, however, was completely insensitive to CGP55845 and instead significantly inhibited by the A1 adenosine receptor antagonist DPCPX.

The effects of DPCPX on LFS-induced depression of polysynaptic EPSPs suggests that adenosine accumulation during 1-Hz stimulation is responsible for a large component of the late phase of LFS-induced depression. Previous studies have shown that activity-dependent increases in adenosine and activation of presynaptic A1 receptors can inhibit transmitter release at Schaffer collateral fiber inputs onto CA1 pyramidal cells, although only during much higher frequencies of synaptic stimulation than those used in our experiments (Brager and Thompson 2003; Manzoni et al. 1994; Mitchell et al. 1993). We find, however, that monosynaptic EPSPs elicited by Schaffer collateral fiber stimulation are not inhibited by short trains of 1-Hz stimulation. Our results thus suggest that recurrent synapses are much more sensitive to the inhibitory effects of adenosine than Schaffer collateral fiber synapses and can be strongly suppressed by activity-dependent increases in extracellular adenosine levels that are below that needed to significantly effect transmission at Schaffer collateral fiber synapses. Although the molecular mechanisms responsible for this differential sensitivity to adenosine are unclear, our results are consistent with the finding that under some experimental conditions ambient levels of extracellular adenosine are sufficient to completely suppress recurrent synapses in the hippocampal CA1 region (Klishin et al. 1995). In addition, although presynaptic GABA_B receptor activation can inhibit transmission at Schaffer collateral fiber synapses (Isaacson et al. 1993; Lanthorn and Cotman 1981; Olpe et al. 1982), we find that pairs of presynaptic fiber stimulation pulses delivered with interpulse intervals of several 100 ms have no effect on monosynaptic EPSPs but induce a GABA_B receptor-mediated paired-pulse depression of transmission at recurrent synapses. Recurrent synapses onto CA1 pyramidal cells thus also appear to be more sensitive to the inhibitory effects of GABA than Schaffer collateral fiber inputs.

Although our results clearly show that A1 adenosine receptor activation has an important role in LFS-induced depression of recurrent synapses a number of important questions remain unresolved. First, the source of adenosine responsible for modulating recurrent synapses during 1-Hz stimulation is unclear. One possibility is that Schaffer collateral fiber stimulation elicits adenosine release from glial cells (Pascual et al. 2005; Serrano et al. 2006). However, Schaffer collateral fiber stimulation-induced adenosine release from glial cells requires GABA release from inhibitory interneurons and subsequent activation of astrocyte GABA_B receptors (Serrano et al. 2006). Thus our observation that blocking GABA_B receptors with CGP55845 has little effect on LFS-induced depression of polysynaptic EPSPs suggests that adenosine release from glial cells is unlikely to have an important role. Other potential sources of adenosine, such as ATP released as a co-transmitter from excitatory synapses and its conversion to adenosine by extracellular 5'-ectonucleotidase (Dunwiddie et al. 1997; Masino et al. 2002) or the transport of adenosine out of pyramidal cells (Brundege and Dunwiddie 1996) appear to be more likely candidates. A second unresolved issue stems from the fact that blocking adenosine receptors only prevents a portion of LFSinduced suppression of polysynaptic EPSPs in CA1 pyramidal cells. This indicates that additional, and as yet unknown, mechanisms also importantly contribute to LFS-induced depression at recurrent synapses. Paired recordings of CA1-CA1 synapses have demonstrated that recurrent synapses exhibit paired-pulse depression (Deuchars and Thomson 1996), suggesting that the probability of transmitter release at these synapse is high. Thus the LFS-induced depression that remains

in the presence of DPCPX may simply reflect depletion of the readily releasable pool of synaptic vesicles. Alternatively, other neuromodulators, such as endocannabinoids (Ohno-Shosaku et al. 2002; Staiker and Mackie 2005) might also be involved. Finally, it is surprising that we observed such robust LFS-induced depression of recurrent synapses during LFS. whereas a previous study of recurrent synapses onto CA1 pyramidal cells found that these synapses exhibit frequencydependent facilitation during 1-Hz stimulation (Radpour and Thomson 1991). Importantly, recurrent synapses were activated via Schaffer collateral fiber stimulation in our experiments while minimal presynaptic fiber stimulation techniques were used in the study by Radpour and Thomason (1991). It thus seems likely that the much larger population of CA1 pyramidal cells activated by the stimulation techniques used in our experiments facilitates accumulation of extracellular adenosine and other neuromodulators involved in LFS-induced depression.

Functional implications of activity-dependent depression at recurrent synapses onto CA1 pyramidal cells

The ability of certain patterns of synaptic activity to strongly depress recurrent synapses without affecting Schaffer collateral fiber synapses has potentially important implications for both normal and pathophysiological processes in the hippocampus. For instance, short-term depression at recurrent synapses may uncouple CA1 pyramidal cells during certain patterns of neuronal activity and thus prevent synchronous bursting that could lead to epileptiform-like activity. Indeed, recurrent synapses between CA1 pyramidal cells are increased in experimental models of epilepsy (Shao and Dudek 2004; Smith and Dudek 2001, 2002), and the density of both A1 adenosine receptors and GABA_B receptors in the hippocampus is reduced in some forms of epilepsy (Glass et al. 1996; Rebola et al. 2003; Straessle et al. 2003). In normal hippocampal function, the selective suppression of recurrent synapses during periods of low-frequency neuronal activity may transform the CA1 region from a sparsely connected recurrent network into a predominately feedforward circuit. LFS-induced and paired-pulse depression at recurrent synapses onto pyramidal cells may thus provide an activity-dependent mechanism for altering the configuration of microcircuits in the hippocampal CA1 region and thereby strongly influence information processing in the hippocampus. Our results indicate that two different neurotransmitters, GABA and adenosine, have a crucial role in this process yet operate over very different temporal domains, i.e., GABA_B receptor activation suppresses recurrent synapses over hundreds of milliseconds, whereas the suppression of recurrent synapses during low-frequency trains of presynaptic action potentials lasting many seconds is largely mediated by adenosine receptor activation. Interestingly, downregulation of presynaptic voltage-gated calcium channels by $\beta\gamma$ subunits of heterotrimeric G proteins is thought to underlie the inhibition of synaptic transmission by a number of neurotransmitters (Tedford and Zamponi 2006), including GABA and adenosine (Wu and Saggau 1994, 1995). Because protein kinase C (PKC)-dependent phosphorylation of calcium channels can inhibit the ability of $\beta\gamma$ subunits to downregulate channel activity (see Tedford and Zamponi 2006 for review), neurotransmitters acting through receptors coupled to PKC activation could strongly inhibit the GABA and adenosine-mediated suppression of recurrent synapses during LFS. Indeed, PKC activation attenuates the ability of adenosine and GABA to inhibit voltage-gated calcium channels in hippocampal neurons (Swartz 1993) and blocks the ability of both adenosine (Thompson et al. 1992) and GABA (Thompson and Gäwiler 1992) to inhibit excitatory synaptic transmission in the hippocampus (Thompson et al. 1992). Thus activity-dependent depression at recurrent synapses between CA1 pyramidal cells may not only represent a mechanism that allows different patterns of neuronal activity to reconfigure the circuitry of the hippocampal CA1 region but also represent a target where modulatory neurotransmitters acting through receptors coupled to PKC activation can act to alter information processing in the hippocampus.

A C K N O W L E D G M E N T S

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$G \mathrel{R} A \mathrel{N} T \mathrel{S}$

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